The performance of HIV rapid antibody detection assays in children

by

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DECLARATION

I, James Jolyon Carey Nuttall, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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CONTENTS

LIST OF ABBREVIATIONS

ARV	Antiretroviral
cART	Combination Antiretroviral Therapy
CI	Confidence Intervals
CT	Cape Town
DNA	Deoxyribonucleic Acid
EIA	Enzyme Immunoassay
EID	Early Infant Diagnosis
ELISA	Enzyme Linked Immunosorbent Assay
GSH	Groote Schuur Hospital
HCT	HIV Counselling and Testing
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
IMCI	Integrated Management of Childhood Illness
Jhb	Johannesburg
LR+	Positive likelihood ratio
LR-	Negative likelihood ratio
Mo	Months
NAAT	Nucleic Acid Amplification Test
NHLS	National Health Laboratory Service
NICD	National Institute of Communicable Diseases
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PD	Presumptive Diagnosis
PMTCT	Prevention of Mother to Child Transmission
PPV	Positive Predictive Value
RCWMCH	Red Cross War Memorial Children's Hospital
RNA	Ribonucleic Acid
RT	Rapid Test
STARD	Standards for Reporting Diagnostic Accuracy
TNA	Total Nucleic Acid
UCT	University of Cape Town

UNICEF	United Nations Children's Fund
VL	Viral load
WB	Western Blot
WHO	World Health Organisation
WITS	University of Witwatersrand

The performance of HIV rapid antibody detection assays in children

Abstract

Background

HIV rapid antibody assays are important for screening children aged <18 months for HIV exposure and children ≥18 months for HIV infection. Limited available data indicate variable performance of different HIV rapid tests in comparison to laboratory HIV antibody assays. The aim of this study was to evaluate the diagnostic accuracy of 6 HIV rapid tests currently used in South Africa for screening children using whole blood.

Methods

A prospective descriptive cross-sectional laboratory study was conducted at two paediatric healthcare facilities in South Africa. Sensitivity and specificity analyses and positive and negative likelihood ratios were performed. The reference standard was the laboratory HIV enzyme-linked immunosorbent assay (ELISA) test and HIV polymerase chain reaction (PCR) test.

Results

Blood samples from 1159 children (896 <18 months of age) with valid HIV ELISA test results were included in the analysis. A total of 5768 HIV rapid tests (4446 in children <18 months of age) were performed. Sensitivity of HIV rapid tests for detecting HIV exposure among children <18 months of age ranged from 38.7% to 94.7%. Four HIV rapid tests attained specificity in excluding HIV exposure among children <18 months of age of >98%. Seroreversion rates were lowest with the Determine rapid test. Three HIV rapid tests (Abon, Advanced Quality, Determine) detected 100% of HIV-infected children <18 months of age, the Reveal, SD Bioline and Insti rapid tests missed 27 (41.5%), 1 (4.5%) and 1 (1.5%) of the HIV-infected children respectively. In children ≥18 months of age, sensitivity of rapid tests for detecting HIV infection ranged from 69.2% to 100% and specificity of all rapid tests was 100%.

Conclusions

None of the 6 HIV rapid tests evaluated achieved both the World Health Organisation recommended sensitivity and specificity standards for any antibody assay used in screening for HIV exposure in children <18 months. The Determine test showed the best overall diagnostic accuracy and is therefore recommended as the preferred screening test for children. Recommendations on the use of specific HIV rapid tests in infants and young children should be based on evaluation of their performance in the population to be tested.

LIST OF TABLES AND FIGURES

TABLES

FIGURES

INTRODUCTION

Background

HIV infection in infants (children <12 months of age) and young children is associated with high rates of morbidity and mortality. [1,2] In 2008, a randomised clinical trial demonstrated that initiation of combination antiretroviral therapy (cART) during early infancy is highly effective in reducing mortality and disease progression. [3] However, by 2010, infants comprised only 19% of all children who initiated cART across 12 Southern African treatment cohorts. [4] One of the obstacles to initiation of cART during early infancy is lack of access to reliable HIV diagnostic testing in resource-limited settings. In South Africa, which has relatively good access to early infant diagnosis (EID), significant progress has been made in prevention, diagnosis and treatment of paediatric HIV infection. In 2012, 83% of pregnant women received antiretrovirals (ARVs) for prevention of transmission, EID coverage reached 72.6%, the transmission rate in infants <2 months of age undergoing testing was 2.4%, and the estimated coverage of cART for HIV-infected children requiring treatment was 63%. [5,6]

HIV antibodies

During pregnancy, physiological transplacental passage of antibodies (Immunoglobulin G, IgG) from the pregnant woman to her foetus occurs. In a pregnant woman with HIV infection, HIV antibodies are transmitted to the foetus whereas the virus itself may or may not be transmitted to the foetus. Maternally derived antibodies may remain in the child's circulation until 18 months of age and in some cases longer. Most commercially available HIV antibody tests, including HIV rapid tests (RTs), are able to detect antibodies in an infant or young child's blood but are unable to distinguish between maternally-derived antibodies and antibodies produced endogenously by a child who has become HIV-infected. [7]

A positive HIV antibody test in a child of unknown HIV status who is <18 months of age usually indicates HIV exposure (maternal HIV infection) and the possibility of

HIV infection in the child which requires further testing using HIV viral detection assays such as polymerase chain reaction (PCR) or p24 antigen testing. HIV-uninfected children eventually lose maternal IgG antibody and revert to seronegative status (seroreversion) since there is no endogenous production of HIV antibody whereas in HIV-infected children, endogenous HIV antibody production occurs and the antibody test remains positive.

A negative HIV antibody test in a child of unknown HIV status who is <18 months of age usually indicates either that the child is not HIV-exposed or that the child is HIV-exposed but has seroreverted. [8] If the child has never been breastfed or not breastfed during the past 6 weeks, the infant can generally be regarded as HIV-uninfected. [9] If the child is still breastfeeding, a negative HIV antibody test does not exclude HIV infection at the time of testing as transmission of HIV infection from mother to child via breastmilk may occur. Diagnosis of HIV infection during breastfeeding generally requires the use of HIV viral detection assays in a child <18 months of age. [9]

Seroreversion

Although there is relatively rapid early decay of HIV-specific maternal IgG in the infant circulation (half-life 28-30 days), there is wide variation in the age at antibody loss (seroreversion) in HIV-exposed-uninfected children ranging between approximately 6 and 24 months in studies using different antibody assays and in different settings. [10-15] Although most studies report the majority of seroreversion occurring between 6 and 15 months, persistence of maternal IgG beyond 18 months of age has been reported. [10,13,15] In a 1994 report, three infants born to HIV-infected mothers were persistently negative on HIV antigen assays and HIV culture (and absolute T-cell numbers and ratios, and quantitative immunoglobulin levels were appropriate for age) but remained HIV antibody positive at 15 months and 18 months before seroreverting at 18.5, 21 and 24.2 months respectively. They also remained clinically well and HIV antibody negative at 3, 4.5 and 5 years of age. [13] In a more recent study investigating the time of seroreversion among 744 infants born to HIV-infected mothers since the introduction of cART for prevention of vertical

transmission, 14% of infants remained seropositive after 18 months, 4.3% after 21 months, and 1.2% after 24 months. Maternal exposure to protease inhibitors was significantly associated with a later age of seroreversion. [15]

There is increasing evidence that early cART and virological suppression can affect the evolution of antibody responses to HIV infection. Failure to develop antibodies or seroreversion occurring in HIV-infected adults treated with cART during acute or early infection and in children following cART initiation during the first few months of life or after maintaining long-term viral suppression on cART has been reported.[16-24] In children, this is particularly relevant in settings where confirmatory HIV testing using viral detection assays is limited or not available and a single viral detection assay or presumptive clinical criteria are used for diagnosis and initiation of cART in children <18 months of age. In this context, the World Health Organisation (WHO) recommends confirmatory testing using HIV antibody assays, including HIV RTs, at the age of 18 months. [9] Studies showing high rates of false-negative HIV antibodies at 18 months - 2 years of age among children who started cART during infancy strongly suggest that HIV antibody tests cannot be used to reliably reconfirm HIV diagnosis in children starting early cART and may lead to uncertainties about the diagnosis of HIV infection and inappropriate discontinuation of cART. [18,20,21,24]

HIV antibody detection assays

Antibodies to HIV can be detected using a variety of different techniques including enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA), rapid test devices and western blot (WB) tests. First-generation HIV ELISA tests became commercially available in 1985. Rapid advances in diagnostic technology led to the development of second and third-generation ELISAs that introduced recombinant protein and synthetic peptide antigens as well as new test formats. These tests had improved sensitivity and specificity, ability to detect HIV-1, HIV-2 and HIV variants, and shortened the interval between infection and detection of antibodies (window period). [25-27]

Laboratory-based ELISA tests are designed for testing large batches of samples applicable to transfusion safety or surveillance programmes. The need for diagnostic testing in individuals in settings with limited laboratory facilities led to the development of highly sensitive and specific rapid tests including agglutination tests, immunocomb tests, immunodot tests using flow through membranes and immunochromatographic membrane tests. These simplified methodologies allow HIV testing to be carried out in primary care facilities by appropriately trained non-laboratory personnel, including counsellors. Health care provider-initiated HIV testing can now include visibility of the test and quick turn-around times, increasing confidence in results and avoidance of clerical errors. Rapid tests allow for testing on whole blood (obtained via finger-prick or heel-prick in infants, or via venesection) or on plasma/serum where centrifugation facilities are available, and some HIV RTs can be used with oral fluid. [25-27] Table 2 summarises important differences between standard laboratory HIV ELISA tests and HIV rapid tests.

Table 1. Comparison of standard laboratory HIV ELISA tests and HIV rapid tests [Adapted from Ref. 27]

Feature	**Standard laboratory HIV ELISA test**	**HIV rapid tests**
Detection method	Detection of HIV antibodies in plasma/serum/oral fluid	Detection of HIV antibodies in whole blood/plasma/serum/oral fluid
Laboratory personnel	Skilled laboratory technician is required	Any health care worker with basic training in performing the test
Equipment	Laboratory equipment including electricity is required	Minimal, usually no additional equipment, reagents, running water or electricity is required
Refrigeration	Required (storage at 2-8°C)	Not required (most can be stored at 2-30°C)
Time to perform	More than 2 hours	1-20 minutes

ELISA = enzyme-linked immunosorbent assay

Western blot tests, initially used mostly for confirmatory testing, are based on a similar methodology to EIA but require sophisticated laboratory equipment and technical expertise. As a result of advances and simplification of other serological assays, WB is no longer essential as a confirmatory test in children or adults.[9]

More recent advances have resulted in the development of combination assays that combine p24 antigen EIAs with traditional antibody EIAs. This allows for simultaneous detection of HIV antigen and antibodies in a single test and further reduces the window period in the detection of early or acute HIV infection. [9]

Since there is a wide range of commercially available HIV RTs, a number of factors must be considered in the selection of which testing kits to incorporate into an HIV testing programme. These factors include availability, cost, location of the testing site, specimen volume required for the assay, training requirements of personnel who will perform the tests, and the diagnostic performance of the test. Although many HIV RTs have undergone diagnostic performance evaluations in comparison to standard laboratory ELISA testing using stored adult plasma samples with known HIV results, performance evaluations in the detection of HIV antibodies in infants and young children is frequently lacking. In 2010, the WHO recommended that HIV antibody assays used for the purpose of clinical diagnostic testing in infants and children should have a minimum sensitivity of 99% and specificity of 98% under quality-assured, standardised and validated laboratory conditions, when used as a screening assay to determine HIV exposure in children <18 months of age or when used as a diagnostic assay in children >18 months of age. [9]

HIV testing strategies using antibody assays

The WHO guidelines (2010) strongly recommend the use of HIV antibody assays to determine the HIV exposure status of all infants with unknown or uncertain HIV exposure at or around birth, the first postnatal visit (4-6 weeks of age) or any child health visit. At any age, infants with signs or symptoms suggestive of HIV infection should have an HIV antibody test and if positive a viral detection assay should be performed. In addition, it is recommended that well HIV-exposed infants should have HIV antibody testing at around 9 months of age (coinciding with the immunisation visit) and infants with a positive HIV antibody test should have a viral detection assay done to identify if they are HIV-infected and require cART. Children aged 18 months or older with suspected HIV infection or HIV exposure should have

HIV antibody testing performed according to the standard diagnostic testing algorithm used in adults. [9]

An HIV testing algorithm describes the combination and sequence of specific HIV testing kits to be used within a given HIV testing strategy. The principle is that a carefully selected combination of different HIV RTs which have validated sensitivity and specificity characteristics (ideally the first test should be highly sensitive (≥99%) and the second test highly specific (≥98%)) used in an appropriate serial testing algorithm will have positive and negative predictive values comparable to or exceeding the traditional laboratory-based ELISA/WB approach. [9] Ideally, the same HIV RTs should be used for HIV testing of adults, children and infants, but in reality many HIV RTs have not undergone adequate performance evaluations in very young children (as screening tests for detection of HIV exposure and for detection of seroreversion amongst HIV-exposed children) in the particular contexts in which they are to be used.

The risk of false-positive results increases the lower the HIV prevalence rate is in the population undergoing testing and the HIV testing algorithm that is selected must take this into account in order to minimise this risk. An HIV prevalence threshold of ≥5% in the target population to be tested is what is recommended by WHO as acceptable to use a two-test serial antibody testing algorithm for the diagnosis of HIV infection in children ≥18 months of age and adults.[9]

In a high HIV prevalence setting (≥5% in the target population), a first positive antibody test result should be confirmed with a second antibody test, using a different test kit on the same specimen. Two positive test results are indicative of a positive HIV antibody result. If test results are discordant (first test positive, second test negative), the specimen should be tested using a third test kit, different from the first two. If this result is negative, the sample is regarded as HIV antibody test negative. If the result of the third test is positive, the individual requires repeat testing three weeks later.

In a low HIV prevalence setting (<5% in the target population), a first positive antibody test result should be confirmed with a second antibody test. If the first and second antibody tests are discordant (first test positive, second test negative), this is regarded as indicative of a negative HIV antibody result. If the first and second antibody tests are positive, the specimen should be tested using a third test kit to confirm a positive result. If the first and second test results are positive and the third is negative, the individual requires repeat testing three weeks later.[9]

According to WHO guidelines, a negative HIV antibody screening test result excludes HIV infection in children ≥18 months of age, and in children <18 months of age indicates either that the infant is not HIV-exposed or that the infant is HIV-exposed but has seroreverted. If there has been no breastfeeding in the past 6 weeks, the infant is regarded as HIV uninfected. The WHO guidelines do not provide specific recommendations on the need for confirmation of negative HIV antibody screening test results in relation to the HIV prevalence in the target population. [9]

Current United States guidelines make a distinction between presumptive exclusion (based on a single negative HIV antibody test result in a non-breastfeeding infant or child ≥6 months of age) and definitive exclusion of HIV infection (based on 2 negative antibody tests from separate specimens obtained at age ≥6 months provided the child has no other clinical or laboratory evidence of HIV infection and is not breastfeeding). [28-30] In recognition of data indicating that children with perinatal exposure aged 18-24 months may have detectable residual maternal HIV antibody, the most recent guidelines recommend that definitive exclusion or confirmation of HIV infection in children in this age group who are HIV antibody positive should be based on HIV nucleic acid testing. Diagnosis of HIV infection in children with non-perinatal HIV exposure or children with perinatal exposure aged >24 months is primarily based on the use of HIV antibody testing although HIV nucleic acid testing may be necessary to diagnose acute HIV infection. [31]

Presumptive clinical diagnosis of HIV infection in children <18 months of age

Laboratory assays that detect the virus or its components allow HIV infection to be definitively diagnosed before 18-24 months of age and facilitate early cART initiation. Nucleic acid amplification techniques such as qualitative or quantitative detection of HIV proviral DNA by PCR, or detection of viral antigen such as p24 antigen have superseded older viral detection techniques such as viral culture. Although point-of-care devices have been developed, most virological detection tests, in particular PCR assays, require expensive laboratory infrastructure and trained personnel and are not available for routine care in all settings where testing is required.

In settings where virological testing is not available, the WHO strongly recommends the use of clinical criteria (2 or more of oral thrush, severe pneumonia, severe sepsis, or a diagnosis of any AIDS indicator condition (*Pneumocystis* pneumonia, cryptococcal meningitis, severe wasting or severe malnutrition, oesophageal candidiasis, Kaposi sarcoma, and extra-pulmonary tuberculosis)) together with HIV antibody testing for the presumptive clinical diagnosis of severe HIV disease needing cART in infants and children <18 months of age. The guidelines indicate that an infant or child who meets these criteria has severe HIV disease and requires immediate cART with the requirement that the diagnosis of HIV infection should be confirmed using age-appropriate testing methods as soon as possible. It is recommended that serological testing should be repeated at 18 months of age to confirm HIV infection in the child but emphasise that access to early virological testing must be made available wherever possible to allow clinicians to implement improved diagnostic algorithms. [9]

A wide variety of observational studies have evaluated the accuracy of different clinical algorithms applied by different levels of healthcare workers in different health care contexts to study populations with different percentages of young infants and different HIV prevalence rates. [32-36] No single clinical diagnostic algorithm has proven to be highly sensitive and specific for diagnosis of HIV infection and they are generally less reliable in infants. [32] In comparison to viral detection assays, the

reported sensitivities of clinical algorithms range between 9% and 89%, specificities between 42% and 99% and positive predictive values between 3% and 95%. [9] Table 1 provides a summary of 2 of these studies. [33,34]

Table 2. The diagnostic performance of the Integrated Management of Childhood Illnesses (IMCI) and WHO Presumptive Diagnosis (WHO-PD) criteria with and without the addition of CD4 criteria for the diagnosis of HIV infection among infants and children <18 months of age in comparison to diagnosis by HIV Deoxyribonucleic Acid (DNA) Polymerase Chain Reaction (PCR) testing

Study	**Sero-positive children <18 mo**	**HIV prevalence rate (DNA-PCR test)**	**Perform-ance of IMCI criteria**	**Perform-ance of WHO-PD criteria**	**Perform-ance of IMCI criteria + CD4% criteria**	**Perform-ance of WHO-PD criteria + CD4% criteria**
Inwani, 2009 [33]	144	80/134 (60%)	Sens: 19% Spec: 96%	Sens: 43% Spec: 88%	Sens: 74% Spec: 43%	Sens: 84% Spec: 41%
Mutesu-Kapembwa, 2010 [34]	299	111/299 (37%)	Sens: 10% Spec: 97%	Sens: 23% Spec: 93%	Sens: 80% Spec: 88%	Sens: 77% Spec: 83%

mo = months, [ ] = reference, Sens = Sensitivity, Spec = Specificity

In South Africa, HIV PCR testing is available for confirmation of HIV infection in all HIV exposed children <18 months of age and is performed by the National Health Laboratory Service (NHLS). National guidelines make provision for HIV antibody testing to be done in children <18 months of age in order to determine whether or not a child is HIV exposed if the mother is not available and there is no record of her HIV status. For children ≥18 months of age, the guidelines recommend that HIV testing should follow the adult testing algorithm. If the HIV RT is positive, as second different HIV RT should be used for confirmation. If the second HIV RT is positive, the child is regarded as HIV positive whereas if the second HIV RT is negative, an HIV ELISA test should be submitted to the laboratory in order to establish the child's HIV status. [37]

The guidelines indicate that a negative antibody detection test at any age excludes infection provided the child was last breastfed ≥6 weeks before the test and has no clinical signs of HIV infection. However, there is no mention of which HIV rapid tests are validated for diagnostic testing purposes in infants and young children. [37] In

2010, the WHO highlighted the need for further research on the performance of different HIV antibody assays in infants and children <2 years of age and this study aims to address this need. [9]

LITERATURE REVIEW ON HIV RAPID TESTS (RT) IN CHILDREN

A directed electronic search of the relevant literature was performed using Pubmed® (accessed via University of Cape Town (UCT) Libraries website). The objective of the literature review was to identify and describe published research on the performance of HIV rapid antibody tests in comparison to laboratory HIV ELISA and HIV PCR testing in children, particularly in infants and very young children. All study designs were included and the search was limited to English language articles. The search strategy included the following search terms: human immunodeficiency virus or HIV, rapid test or rapid testing or rapid antibody test, infant, child, early diagnosis or early infant diagnosis, diagnostic accuracy, seroreversion. Article abstracts were scanned and relevant full text articles (7) were downloaded via UCT Libraries (http://www.lib.uct.ac.za/). Additional articles (5) were obtained by cross-referencing the articles included and relevant conference abstracts (2) were also included. The literature review was not continued beyond the end of December 2014.

Children older than 18 months of age

Evaluations of the performance characteristics and diagnostic accuracy of HIV RTs in comparison to laboratory HIV ELISA and HIV virological detection assays have mostly involved adult populations. Some variability in the performance of different HIV RTs used under field conditions has been reported, however most third-generation HIV RTs have reported sensitivities and specificities of between 94 and 100% and 88 and 99.4% respectively in comparison to laboratory HIV ELISA.[27,38-40]

In children older than 18 months of age, the performance of HIV RTs for the diagnosis of HIV infection has generally been reported as equivalent to adults although there are a very limited number of published studies and HIV RT assays evaluated.

De Baets et al. evaluated alternative tests and strategies to simplify paediatric HIV screening in an African district hospital in 2003.[41] The study included 788 children older than 18 months of age (mean age 6.7 ±3.3 years) enrolled at 7 sites. The

reference testing algorithm for children older than 18 months of age incorporated three laboratory HIV antibody assays: Vironostica HIV Uni-Form II Plus O (bioMe´rieux bv, Boxtel, The Netherlands), Enzygnost Anti-HIV 1/2 Plus (Dade Behring Marburg GmbH, Marburg, Germany), and INNO-LIA HIV (Innogenetics S.A., Ghent, Belgium) on the same sample of venous plasma. An HIV-negative status was based on the first test or two of the three tests being negative. An HIV-positive status was based on two positive tests. An HIV viral load (VL) test (Cobas AmpliPrep/Amplicor HIV-1 [V1.5]; Roche, Branchburg, N.J.) was performed on each HIV-positive patient and was above 50 copies/ml in all cases, confirming the diagnosis of HIV infection. The HIV prevalence rate in the study population was 3.9%.

The proposed alternative screening strategy used serial HIV RTs selected because of characteristics that made them suitable for use in an African setting and performed on the same sample of capillary blood stored in ethylenediaminetetraacetic acid (EDTA) tubes. The Determine HIV-1/2 test (Abbott Laboratories, Tokyo, Japan) was done as the initial screening test. If the Determine test was negative, the patient was regarded as HIV-negative. If the Determine test was positive, the InstantScreen Rapid HIV-1/2 test (Gaifar GmbH, Potsdam, Germany) was performed and if positive the patient was regarded as HIV-positive. This differs from WHO recommendations which propose the use of a third HIV antibody test to confirm two positive results in a low HIV prevalence setting.[9] According to the testing algorithm, if the Determine test result was indeterminate (1.3% of tests done), the InstantScreen test was done and if positive this was followed by the Uni-Gold HIV test (Trinity Biotech PLC, Bray, Ireland). However, all the indeterminate Determine tests were negative on further testing with InstantScreen.

The alternative screening strategy had a sensitivity of 100% (95% confidence interval (CI) 99.5 to 100%) and a specificity of 100% (95% CI 99.5 to 100%) in comparison to the reference testing algorithm. Additional analysis of the Determine test results showed that there were no false-negative results and 2 false-positive results (excluding indeterminate cases) among the 788 cases tested.

The authors concluded that the use of a testing algorithm based on serial HIV RTs performed on capillary blood stored in EDTA tubes (obtained by finger-prick using glucolet capillary phlebotomy devices as opposed to the reference testing algorithm which used venous plasma obtained by venesection and centrifugation) instead of laboratory-based HIV ELISA assays performed as well in children older than 18 months of age as in adults. This approach could simplify HIV diagnosis in children allowing same-day receipt of test results at lower costs and facilitate decentralisation of HIV screening. The study was performed in a low HIV prevalence setting (3.9%) which may have led to the increased risk of false-positive results (although still low at 0.3%) and therefore warranted repeating in a higher HIV prevalence setting. The authors also highlighted the value of HIV RTs in excluding HIV infection as there were no false-negative Determine test results although it is important to establish whether breastfeeding has been stopped.

Sherman et al. 2012, evaluated the diagnostic performance of 5 HIV RTs (First Response HIV Card 1-2.0 (Premier Medical Corporation, Daman, India); Pareekshak HIV-1/2 Triline Card (Bhat Biotech, Bangalore, India); Abbott Determine HIV-1/2 (Abbott Laboratories, North Chicago, IL); Smart Check HIV-1/2 (World Diagnostics, Inc., Miami, FL) and Insti HIV-1 (BioLytical Laboratories, British Columbia, Canada) using whole blood in a multisite cross-sectional HIV diagnostic study in South Africa which included children older than 18 months of age.[42] The HIV prevalence rate amongst the children older than 18 months of age was much higher than in the De Baets study and ranged from 43-64% amongst the sub-groups tested with different HIV RTs. Children receiving cART were excluded.

In comparison to third and fourth generation laboratory HIV ELISA tests (HIV-1/HIV-2 III Plus, IMx System and AxSYM HIV Ag/AbCombo, respectively, Abbott Diagnostics Division, Wiesbaden, Germany), all 5 HIV RTs were 100% sensitive in detecting HIV-infected children. First Response, Pareekshak and Smart Check were 100% specific in excluding HIV infection in uninfected children over 18 months of age, but the Determine and INSTI RTs each gave one false-positive result (Table 3.) The authors concluded that any one of the 5 HIV RTs could be used as a screening

test for HIV diagnosis in children over 18 months of age and First Response, Pareekshak or Smart Check would be suitable confirmatory tests.

Table 3. Sensitivity and specificity of various HIV rapid tests in comparison to standard laboratory HIV ELISA for diagnosis of HIV infection in children >18 months of age

HIV Rapid Test	**Determine**	**First Response**	**Pareekshak**	**Smart Check**	**Insti**
Sherman, 2012 [42]	N=111	N=114	N=95	N=103	N=28
Sensitivity % (95% CI)	**100** (94.9-100)	**100** (95-100)	**100** (93.9-100)	**100** (94.3-100)	**100** (75.8-100)
Specificity % (95% CI)	**97.5** (87.1-99.6)	**100** (91.4-100)	**100** (90.4-100)	**100** (91-100)	**93.8** (71.7-98.9)
HIV prevalence (%)	64	64	62	62	43

95% CI = 95% confidence interval, [ ] = reference

Children younger than 18 months of age

In this age group, the performance of HIV RTs both for the detection of HIV exposure (indicating the need for further virological testing if available) as well as for the exclusion of HIV infection (either where the maternal HIV status is not known or by demonstrating seroreversion in HIV-exposed children) requires evaluation. The sensitivity of HIV RTs in children <18 months of age is the ability to detect HIV exposure as compared to laboratory HIV ELISA testing. The specificity of HIV RTs is the ability to correctly identify HIV-uninfected children (including seroreversion). The ability of an HIV RT to not miss any HIV PCR positive children <18 months of age is another important criterion to be evaluated.

Detection of HIV exposure and exclusion of HIV infection by HIV RT

Table 4 summarises the 2 most important studies, both performed in South Africa, which evaluated the performance of various HIV RTs in children ≤18 months of age.

Table 4. Sensitivity and specificity of various HIV RTs in comparison to standard laboratory HIV ELISA for detection of vertical HIV exposure in children ≤18 months of age

HIV Rapid Test	Determine	First Response	Insti	Ora-Quick	Pareek-shak	Smart Check	Uni-gold
Sherman, 2008 [43] (tested at 1.5, 3, 7 & 12 mo of age) Sample size **Sensitivity %** (95% CI) HIV prevalence: 18.1%	N=289 **99.7** (99-100.3)	N=288 **79.9** (75.5-84.2)	N=273 **70.3** (65.3-75.4)	N=286 **75.5** 70.9-80.2)	N=284 **73.2** (68.4-78.1)	N=284 **81.3** (77.1-85.6)	N=289 **72.7** (67.8-77.5)
Sherman, 2012 [42] (≤18 mo of age) <u>Sensitivity:</u> Sample size **Sensitivity %** (95% CI) HIV prevalence <u>Specificity:</u> Samples size: 138 **Specificity %** (95% CI)	N=580 **95.5** (93.5-96.9) 18.8 **99.2** (95.6-99.9)	N=596 **84.6** (81.4-87.2) 18.8 **100** (97.3-100)	N=254 **95.7** (92.4-97.6) 12.6 **96.2** (87.0-98.9)	N/E	N=564 **86.3** (83.3-88.9) 18.4 **100** (97.2-100)	N=442 **88.0** (84.7-90.7) 17.4 **100** (96.7-100)	N/E

95% CI = 95% confidence interval, N/E = not evaluated, [ ] = reference, mo = months

Sherman et al. 2008, evaluated the performance of 7 different HIV RTs in comparison to laboratory HIV ELISA at four ages (1.5, 3, 7 and 12 months) during infancy for detecting HIV exposure (sensitivity) and seroreversion (specificity).[43] The study used 2266 stored plasma and serum samples from 116 HIV-exposed infants of known HIV status (21 HIV-infected and 95 HIV-uninfected) who had been enrolled in an HIV diagnostic study in South Africa. All infants had only been exposed to single-dose nevirapine at delivery and no other ARVs. The definitive HIV status of each infant was determined with at least 2 HIV DNA PCR tests (Roche Amplicor version 1.5, Roche Molecular Systems, Basel, Switzerland) during study visits at 6 weeks, and 3-, 7- and 12 months of age. Surplus plasma and serum from samples submitted for HIV PCR and HIV ELISA tests (HIV-1/HIV-2 III Plus,

IMx System, Abbott Diagnostics Division, Wiesbaden, Germany) respectively were stored at −70°C.

The RTs evaluated were selected as follows: Abbott Determine HIV-1/2 (Abbott Laboratories, Illinois, USA) and UniGold (Trinity Biotech, Co Wicklow, Ireland) because of published good performance in children older than 18 months; First Response HIV Card 1-2.0 (Premier Medical Corporation, Daman, India), Smart Check (Globalmed, Vancouver, USA) and Pareekshak HIV-1/2 Triline Card (Bhat Biotech, Bangalore, India) because they had been validated in adults and were being used in South Africa at the time of the study; Oraquick test (OraSure Technologies, Pennsylvania, USA) because additional experience using plasma and serum samples to complement previously published data on oral fluid testing was needed; and Insti HIV-1 RT (BioLytical Laboratories, British Columbia, Canada) because the testing time with this RT was 1 minute instead of the usual 10-20 minutes.

From the 2266 samples, 1993 had HIV ELISA positive results and 273 (all from 12-month-old infants) had HIV ELISA negative results. Not all infants had sufficient stored sample to perform all 7 HIV RTs but each HIV RT was evaluated on a median of 40 (range 34-42) and 285 (range 277-287) samples from HIV-infected and HIV-uninfected infants respectively. Weakly positive HIV RT results were reported as positive. If HIV RT and HIV ELISA results did not concur, the HIV RT was repeated and if the results remained discordant, the HIV ELISA test was repeated on the stored sample to pick up recording errors or antibody loss during storage.

An important finding of this study is that the 7 HIV RTs evaluated did not perform equally in the detection of HIV-exposure during the first 12 months of age. During the first 3 months of age, the sensitivity of all the HIV RTs was similar to HIV ELISA but thereafter only 1 HIV RT, Determine, maintained sensitivity (99.7%) within the WHO recommended range of 99-100% for HIV screening assays. The overall sensitivity of the other HIV RTs ranged between 70.3 and 81.3%.

There were 275 samples from the 21 HIV-infected infants and 271 (98.5%) of these samples gave a positive HIV RT result. The 4 false-negative results were from 2

infants who had available stored sample only at 3 months of age. Both infants were negative on Unigold and OraQuick testing. One of the infants had sufficient sample for retesting and the repeat tests remained negative. There was insufficient sample to repeat the HIV ELISA test. The remainder of the negative HIV RT results among HIV ELISA positive samples were from 95 HIV- exposed but uninfected infants. Unigold, First Response and Insti each had a negative result at 3 months of age or younger which would have resulted in these 3 HIV-exposed uninfected infants being undetected. None of the HIV RTs detected seroreversion at 3 months of age but by 7 months the different sensitivities of the 6 HIV RTs other than Determine when compared to HIV ELISA was seen.

When comparing the HIV RT results to the HIV ELISA negative results at 12 months of age, there were no false-positive HIV RT results except for 7 infants who had seroreverted according to ELISA but remained positive on the Determine RT emphasising the high sensitivity of this test.

The specificity of the HIV RTs (ability to correctly exclude HIV infection) was assessed on 1991 samples from 95 HIV-uninfected infants. At 12 months of age, 40% of these HIV-uninfected infants still had positive HIV ELISA tests, 50% were still positive on the Determine RT, and only 0-6% of the remaining 6 HIV RTs were still positive.

Another important finding of this study is that the HIV ELISA test was positive in all HIV-exposed infants at 7 months of age or younger but by 12 months of age had a negative predictive value (NPV) of 100%. Apart from Unigold and OraQuick which didn't detect 2 HIV-infected infants at 3 months of age, all the other HIV RTs had a NPV of 100%. This finding is of clinical significance as it implies that a negative HIV RT selected on the basis of the performance described in this study may be used to accurately exclude HIV infection in an infant.

As would be expected, the positive predictive value (PPV) of HIV ELISA or HIV RT remains low (<20%) at 3 months of age or younger. By 7 months of age, the PPV

of HIV ELISA and Determine are still low (median 16%) but the median PPV of the other HIV RTs increased to 41% (at 7 months) and 80% (at 12 months).

The authors concluded with the observation that the selection of which commercially available HIV RTs should be used for HIV screening of infants must be based on evaluation of the HIV RTs in the infant population and not extrapolated from adult data as not all HIV RTs perform equally across infancy. They also highlighted the need for further evaluations of HIV RT performance in infants on whole blood rather than stored plasma and in less-controlled field conditions rather than laboratory conditions.

In a subsequent study, Sherman et al. evaluated the accuracy of 5 HIV RTs for detecting HIV exposure in early infancy and excluding HIV infection in HIV-exposed infants using whole blood.[42] Blood samples were obtained from children enrolled in a multisite cross-sectional study at 3 university hospitals, 6 primary healthcare clinics and an adoption centre in Johannesburg, South Africa. Children receiving ART were excluded. The HIV RTs initially included were First Response HIV Card 1-2.0 (Premier Medical Corporation, Daman, India) and Pareekshak HIV-1/2 Triline Card (Bhat Biotech, Bangalore, India), both of which were in use in South African public health-care facilities at the time of the study; and Abbott Determine HIV-1/2 (Abbott Laboratories, North Chicago, IL), previously shown to be highly sensitive using plasma. During the study, the Smart Check HIV-1/2 (World Diagnostics, Inc., Miami, FL) RT was added and later Insti HIV-1 (BioLytical Laboratories, British Columbia, Canada) was also included because of its short testing time of 1 minute. The HIV RT s were performed in the order described. Laboratory-based testing included third and fourth generation HIV ELISA (HIV-1/HIV-2 III Plus, IMx System and AxSYM HIV Ag/Ab Combo, respectively, Abbott Diagnostics Division, Wiesbaden, Germany) and the HIV-1 DNA PCR assay (Roche Amplicor HIV-1 DNA PCR version 1.5, Roche Molecular Systems, Basel, Switzerland). Two reactive HIV ELISA results defined HIV exposure in children aged 18 months or less. The HIV infection status of children less than 18 months of age was determined by the DNA HIV PCR assay. The results of the various HIV RTs were compared against these reference standards.

There were 737 children ≤18 months of age and the prevalence of HIV infection amongst the children ranged from 7.5 – 37% across different age groups. The sensitivity of the HIV RTs for detection of HIV exposure ranged from 84.6 – 95.7% with Insti and Determine performing the best. The sensitivity was higher in children <3 months of age than in older children. Importantly, not all HIV-infected children were detected by the HIV RTs. Among infants >8 months of age, all the HIV RTs except the First Response test, were able to accurately exclude HIV infection if combined with a clinical assessment for overt clinical features of HIV infection (collected at enrolment). In infants <3 months of age, Determine showed the highest sensitivity for detecting HIV exposure of 99.3%, but it missed 1 HIV-infected infant, whereas Insti and Smart Check showed lower sensitivities for detecting HIV exposure but detected all of the HIV-infected children. First Response and Pareekshak showed the lowest sensitivity for detecting HIV exposure in infants <3 months of age, and they missed the greatest number of HIV-infected infants.

Among 138 ELISA-negative children ≤18 months of age, there was 1 false-positive Determine (specificity 99.2% [95% CI: 95.6 – 99.9]) and 2 false-positive Insti (specificity 96.2% [95% CI: 87.0 – 98.9]) results. First Response, Preekshak and Smart Check were 100% specific.

Regarding seroreversion (negative HIV RT result in HIV-exposed [ELISA positive] but uninfected [PCR negative] children), rates were less than 20% below 4 months of age, 50% by 4-6 months of age with all HIV RTs except Determine, and 100% after 8 months of age with all of the HIV RTs except Determine. Insti did not miss any HIV-infected children and was the most accurate HIV RT for detecting seroreversion after 6 months of age.

This study showed that in the first 3 months of life, Determine was the only HIV RT of the 5 evaluated which attained the WHO recommended sensitivity cut-off of ≥99% for clinical diagnostic testing, in this context used as a screening assay to detect HIV exposure. For all children ≤18 months of age, none of the HIV RTs achieved a sensitivity for the detection of HIV exposure of >96% and therefore failed to meet WHO recommendations. This can be explained by the increasing rate

of seroreversion as children approach 18 months of age. In this study, all 5 HIV RTs detected 100% seroreversion in children >10 months of age. Reliable detection of seroreversion well before 18 months of age is desirable to avoid the risk of incorrectly labelling a child as HIV-infected by HIV RTs at ≥18 months of age. The authors recommended that further evaluation of the sensitivity of HIV RTs for detection of HIV exposure in the age group 4-18 months was warranted as this study included relatively low numbers of children in this age group.

The specificity for detecting HIV exposure attained the WHO recommendation of ≥98% for all of the HIV RTs except Insti (96.2%). The authors also noted that their results highlighted the reduced sensitivity of HIV RTs on whole blood in this study compared to stored serum or plasma in a previous study. This is an important consideration in the selection of HIV RTs for use in paediatric testing programmes as capillary blood (whole blood) obtained from finger- or heel-prick sampling may be more practical than venesection and centrifugation in primary care settings.

In an unpublished Kenyan study, the performance of Determine and Bioline RTs was assessed on whole blood of 9-month old HIV-exposed infants.[44] In this study, the seroreversion rate was 90% but amongst the HIV-infected infants, the detection rate using Determine and Bioline was 72% and 83% respectively, significantly lower than the WHO recommended threshold of 99%.

In another unpublished study from Botswana, Determine RT detected 100% of HIV-infected infants less than 18 months of age but the Unigold RT detected only 87%.[45]

Detection of HIV-exposed and –infected children by HIV RT

An important role for HIV RTs in children <18 months of age is the ability to detect and not to miss HIV-exposed (HIV ELISA-positive) and –infected (HIV PCR-positive) children. Table 5 summarises 3 important studies that have evaluated the performance of various HIV RTs in detecting HIV-exposed and –infected children ≤18 months of age.

Table 5. Summary of published studies on the performance of various HIV rapid tests for detection of HIV-exposed and –infected (HIV ELISA+ HIV PCR+) children ≤18 months of age

HIV Rapid Test (RT)	Determine	First Response	Insti	Pareek-shak	Smart Check	Stat-Pak
De Baets, 2005 [41] (<18 mo of age)						
Sample size	116	N/E	N/E	N/E	N/E	N/E
% (N) **HIV PCR+ detected by HIV RT alone**	**100** (116/116)					
Menzies, 2009 [46] (1.5-18 mo of age)						
Sample size	58	N/E	N/E	N/E	N/E	144
% (N) **HIV PCR+ detected by HIV RT alone**						
1.5-3 mo	**92.6** (25/27)					**100** (34/34)
>3-6 mo	**88.9** (8/9)					**89.3** (25/28)
>6-9 mo	**100** (6/6)					**83.3** (15/18)
>9-12 mo	**100** (6/6)					**93.3** (28/30)
>12-18 mo	**90** (9/10)					**97.1** (33/34)
All age groups	**93.1** (54/58)					**93.8** (135/144)
Sherman, 2012 [42] (≤18 mo of age)						
Sample size	109	112	32	104	77	N/E
% (N) **HIV PCR+ detected by HIV RT alone**						
≤2 mo	**100** (24/24)	**91.3** (21/23)	**100** (15/15)	**95.5** (21/22)	**100** (22/22)	
>2-4 mo	**95.8** (23/24)	**92.3** (24/26)	**100** (6/6)	**79.2** (19/24)	**76.5** (13/17)	
>4-6 mo	**100** (16/16)	**87.5** (14/16)	**100** (2/2)	**100** (15/15)	**100** (10/10)	
>6-8 mo	**83.3** (10/12)	**66.7** (8/12)	**100** (4/4)	**75** (9/12)	**75** (6/8)	
>8-10 mo	**90** (9/10)	**90** (9/10)	**100** (2/2)	**90** (9/10)	**100** (6/6)	
>10-12 mo	**100** (7/7)	**77.8** (7/9)	**100** (2/2)	**100** (6/6)	**100** (6/6)	
>12-18 mo	**100** (16/16)	**100** (16/16)	**100** (1/1)	**100** (15/15)	**100** (8/8)	
All age groups	**96.3** (105/109)	**88.4** (99/112)	**100** (32/32)	**90.4** (94/104)	**92.2** (71/77)	

PCR= polymerase chain reaction, N/E = not evaluated, [ ] = reference, mo = months

De Baets et al. reported that among 116 samples from children <18 months of age (mean age 9.7 ±4.9 months) with an HIV prevalence of 8.5%, there were no false negative (only three false-positive) Determine RT results using DNA PCR performed on dried blood spots stored on filter paper as the reference standard and with confirmation of all HIV PCR positive samples with HIV viral load testing.[41]

Menzies et al. investigated the cost-effectiveness of incorporating initial screening with HIV RTs into a conventional infant HIV testing algorithm as a means of screening-out HIV-uninfected infants and thereby reducing the need for costly HIV virological detection assays.[46] The HIV RTs used in the modified testing algorithm were either HIV 1/2 Determine (Abbott Laboratories, Abbott Park, IL) or HIV 1/2 Stat-Pak (Chembio Diagnostics, Medford, NY), both of which were being used in Uganda's adult HIV RT algorithm, and the conventional testing programme used Roche Amplicor HIV-1 DNA-PCR v1.5 (Roche Diagnostic Systems, Inc., Branchburg, NJ). HIV prevalence, RT sensitivity and specificity, and cost were analysed from a cohort of 788 HIV-exposed children aged 1.5 to 18 months who were attending 2 postnatal HIV screening programmes in Uganda. There were 202 HIV PCR positive children (HIV prevalence 25.6%), 58 were tested with Determine and 144 with Stat-Pak. Sensitivity in comparison to the HIV PCR result was 93.1% (95% CI: 83.3%–98.1%) and 93.8% (95% CI:88.5%–97.1%) for Determine and Stat-Pak respectively. The authors ascribed the reduced sensitivity of the HIV RTs to the high HIV incidence in a breastfeeding population and the possibility of infants being in the window period for HIV infection. The cost-effectiveness model estimated that incorporation of these 2 HIV RTs into screening excluded HIV infection from 3 months of age or older in a more cost-effective manner than virological assays.

In the study by Sherman et al. 2012, one of the objectives was to compare the performance of 5 HIV RTs in detection of HIV PCR positive children ≤18 months of age using whole blood and to assess whether the HIV RTs would miss any HIV-infected children. [42] All of the HIV RTs except Insti missed HIV-infected infants of all ages and there was considerable variability by HIV RT and across different age groups (Table 5).

Buchanan et al. also assessed the utility of HIV RTs to exclude HIV infection among infants and children ≥2 to <18 months in a low-resource setting in Tanzania where HIV nucleic acid amplification tests (NAATs) are not routinely available.[47] The study included children who had been admitted to hospital with an acute febrile illness and enrolled in an observational study on invasive bacterial infection. At the time of enrolment in the initial study, Capillus (Trinity Biotech, County Wicklow,

Ireland) and Determine (Abbott Laboratories, Abbott Park, IL, USA) RTs were performed at the bedside by nurses trained and supervised by the study medical team. Children in whom both HIV RTs were positive underwent HIV NAAT (Abbott Real-Time m2000 System, Abbott Molecular, Illinois, USA), children with discordant HIV RT results had HIV ELISA (Vironistika Uniform II Plus-O Test, bio-Mérieux, NC, USA), but no further testing if negative on HIV ELISA. Children with two negative HIV RT results also had no further testing at the time of the initial study.

The present study tested samples without prior HIV NAAT from the original 2006 to 2007 study that had been in storage at −80 ◦C for 4–5 years, which included all children with concordant negative HIV RT or discordant HIV RT with negative HIV ELISA, and samples with initially discordant HIV RT results and undetectable HIV-1 RNA PCR. HIV-infected was defined as a single positive HIV-1 RNA PCR > 400 copies/ml; HIV uninfected was defined as a single negative undetectable HIV-1 RNA PCR. The HIV prevalence was 7% amongst the mothers and 3.4% amongst the 1602 enrolled children. The study did not collect data on maternal or infant cART although the authors state that since the study was done in 2006/2007 it is unlikely that many of the children in this age range would have been diagnosed with HIV infection and on cART.

The performance of the HIV RTs used alone and in parallel for the exclusion of HIV infection in comparison to HIV NAAT was evaluated. The study found that all children (n=1526) with 2 negative HIV RTs were HIV negative by HIV NAAT and all children (n=46) with 2 positive HIV RTs were HIV positive by HIV NAAT. Overall, 2 negative HIV RTs performed in parallel had a NPV (95% CI) for HIV infection of 100% (99.7-100%), and a single negative RT had a NPV of 99.9% (99.6-100%) (Determine) and 99.5% (99-99.7%) (Capillus). Sensitivity and specificity were ≥99% and >98% respectively across all age brackets between ≥2 months and <18 months. Sensitivity and specificity for a single HIV RT was 98.2% (90.4-99.7%) and 99% (98.4-99.4%) respectively for Determine, and 85.5% (73.8-92.4%) and 99.6% (99.2-99.8%) respectively for Capillus. In summary, no infected child had 2 negative HIV RTs, no uninfected child had 2 positive HIV RTs, and

discordant results occurred in <2% of those tested. This study highlighted the valuable role that HIV RTs can play in excluding HIV infection in children aged <18 months with much lower cost and complexity than HIV NAAT.

HIV RT on oral fluid in children

Oral fluid samples may be tested for HIV antibodies and a number of assays have been developed for laboratory ELISA or HIV rapid testing. Oral fluid sampling is less invasive than blood sampling and may be more acceptable to parents whose infants are undergoing HIV screening. For healthcare workers less skill is required, there is lower risk of occupational exposure to HIV infection and it is less time-consuming than blood sampling.

OraQuick Advance Rapid HIV-1/2 Antibody Test (OraSure Technologies, Bethlehem, PA), an oral fluid rapid test, and OraSure, an oral fluid collection device that is licensed for use with the oral fluid Vironostika Microelisa system (Organon Teknika Corp., Durham, NC) have been found to have sensitivities between 93.3 and 100% and specificities of 99.2 to 100% in adult studies.[48,49] These two tests were evaluated in children 11 to 18 months of age for the detection of seroreversion as a means of excluding HIV infection in HIV-exposed children.[50] Sensitivity was 87% (OraQuick) and 95% (OraSure) and specificity was 97% (OraQuick) and 93% (OraSure). However, both tests did show NPV of >99% and could potentially be used to detect seroreversion earlier than laboratory HIV ELISA.

In a more recent study involving infants <6 months of age, OraQuick, OraSure, and another oral fluid antibody test (Calypte AWARE HIV-1/2 OMT Antibody Test (Calypte Biomedical Corporation, Portland, OR) achieved sensitivities <80% and the authors stated that oral fluid rapid tests cannot currently be recommended above blood HIV RTs to screen for HIV exposure in early infancy.[51]

RATIONALE FOR DOING THE STUDY

Although previous and current South African NDOH guidelines for the management of HIV in children make provision for the use of HIV RTs to assess HIV-exposure in children <18 months of age, uncertainty about the interpretation of HIV RT results in children <18 months of age or lack of data validating the performance of HIV RTs in infants and young children result in the HIV PCR test frequently being the only HIV test used in children <18 months of age .[52,53] It would be preferable for the same HIV RTs to be used for both HIV screening in infants and young children and HIV diagnosis in adults as this would reduce costs, training and complexity for public health programmes. This would also facilitate the incorporation of infants and young children into health care provider-initiated HIV counseling and testing (HCT) campaigns. In addition, HIV RTs that reliably and accurately detect seroreversion early but are also sensitive enough not to miss HIV-exposed, infected infants reduce the need for expensive HIV PCR tests and allow for same-day results. Based on previous studies, HIV RTs could potentially distinguish virtually all HIV-exposed uninfected from HIV-exposed infected infants from 8-10 months of age and possibly as early as 6-8 months of age. [42,43]

This study set out to evaluate the performance in children of HIV RTs currently in use in the South African public sector for both adult and paediatric HIV testing. The 2009-2011 South African National Department of Health (NDOH) tender included the following HIV RTs: Advanced Quality One Step Anti HIV (1&2) (InTec Products Inc.); SD Bioline HIV 1/2 3.0 (Standard Diagnostics, Inc.); G. Ocean (additional details not available), First Response HIV 1-2.0 Card test (Premier Medical Corporation Ltd.), and Abbott Determine HIV-1/2 (Abbott Laboratories).

Existing data indicate that not all HIV RTs perform equally well in HIV-exposed infants and children <18 months of age. Among the HIV RTs that have been evaluated using the laboratory HIV ELISA test as the standard for comparison, only the Determine RT has shown sensitivity for the detection of HIV exposure that meets the minimum requirement of 99% recommended by WHO for an HIV antibody screening assay in infants. [43] The sensitivity of various other HIV RTs have been as

low as 70%. [42,43] Since there are very few published studies, public health programmes have little evidence on which to base their choice of HIV RTs to be used in infancy and run the risk of under-diagnosing HIV-exposure leading to missed treatment opportunities for HIV-infected children. WHO has highlighted that assessing the performance of different HIV serological assays in infants and young children is an outstanding issue requiring further research.[9]

RATIONALE FOR SELECTING RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN, WESTERN CAPE PROVINCE, SOUTH AFRICA AS ONE OF THE STUDY SITES

In the South African public health sector, procurement of HIV RTs is via a national tender process with subsequent allocation to provinces. The specific HIV RTs allocated to provinces for screening and confirmation of HIV infection varies by province and does not take into account validation for paediatric testing.

However, in June 2008, based on published scientific literature indicating higher sensitivity of the Determine RT for detection of HIV exposure in infants in comparison to other available HIV RTs, the Western Cape Province Department of Health introduced a new HIV testing algorithm for children. [43] According to the algorithm (Figure 1), children with unknown HIV status are screened using the Determine RT. If the Determine RT result is negative, the child is reported as sero-negative. If the Determine RT result is positive, the specimen is tested with a second test. For children <18 months of age, the confirmatory test is the HIV PCR test and for children >18 months of age, the confirmatory test was the laboratory HIV ELISA test.

Since 2008, all HIV testing at Red Cross War Memorial Children's Hospital (RCWMCH), a paediatric tertiary referral hospital in Cape Town has been carried out in accordance with the Western Cape Province HIV testing algorithm. HIV testing takes place in the Haematology laboratory that is quality-accredited and managed by the National Health Laboratory Service (NHLS). In the routine HIV testing algorithm, testing is usually performed on EDTA blood samples. Ideally, 2 EDTA microtainer tubes with ±500µl of blood in each tube are submitted to the laboratory but this is dependent on the volume of blood obtained by the clinician performing venesection on the child. The sample (first tube) undergoes centrifugation at 2500 revolutions per minute for 10 minutes. The Determine HIV RT is then performed on plasma according to the manufacturer's instructions in the package insert. A child whose blood sample tests negative with the Determine HIV

RT is reported as HIV-uninfected although the clinician is required to interpret the result in the context of infant feeding as described in Figures 1. In children who test positive with the Determine RT, the same sample or a second EDTA sample if available is forwarded to the virology laboratory at Groote Schuur Hospital (GSH) for the TNA PCR test (COBAS Ampliprep/COBAS TaqMan HIV-1 Qual test (Roche) if the child is <18 months of age or HIV ELISA test (Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA) if the child is ≥18 months of age. The Behring Enzygnost Anti HIV1/2 Plus has a sensitivity of 100% and specificity of 99.3-100% in relation to comparable laboratory assays in studies on adult samples performed at four different centres in Europe, North America and Africa according to the manufacturers.[54] Data on paediatric samples is not available.

Between January and December 2010, approximately 5226 children <15 years of age underwent HIV rapid testing at RCWMCH (personal communication, RCWMCH NHLS laboratory). Table 6 summarises the results of children undergoing HIV testing at RCWMCH during 2010.

The combination of a quality-assured laboratory infrastructure that implemented a standardised HIV testing algorithm together with relatively high numbers of children across all age categories undergoing HIV RT and HIV PCR testing suggested that RCWMCH represented a reliable, efficient and cost-effective location to undertake a study on the performance of HIV RTs in children. However, it was acknowledged at the outset that the duration of the study would depend on accessing adequate volumes of leftover blood samples in order to perform the HIV RTs being evaluated as well as adequate numbers of HIV-infected children to enable meaningful statistical analysis.

At the same time as the Cape Town study was being performed, a similar study evaluating the same HIV RTs on leftover whole blood samples from children undergoing HIV PCR or HIV viral load testing took place in the Department of Molecular Medicine and Haematology, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa under supervision of Professor Gayle Sherman. Professor Sherman also assisted with the development of the RCWMCH

study. Combining the 2 study datasets into a single larger dataset allowed for a more powerful statistical analysis to be performed.

Figure 1. HIV testing algorithm, Western Cape (2008)

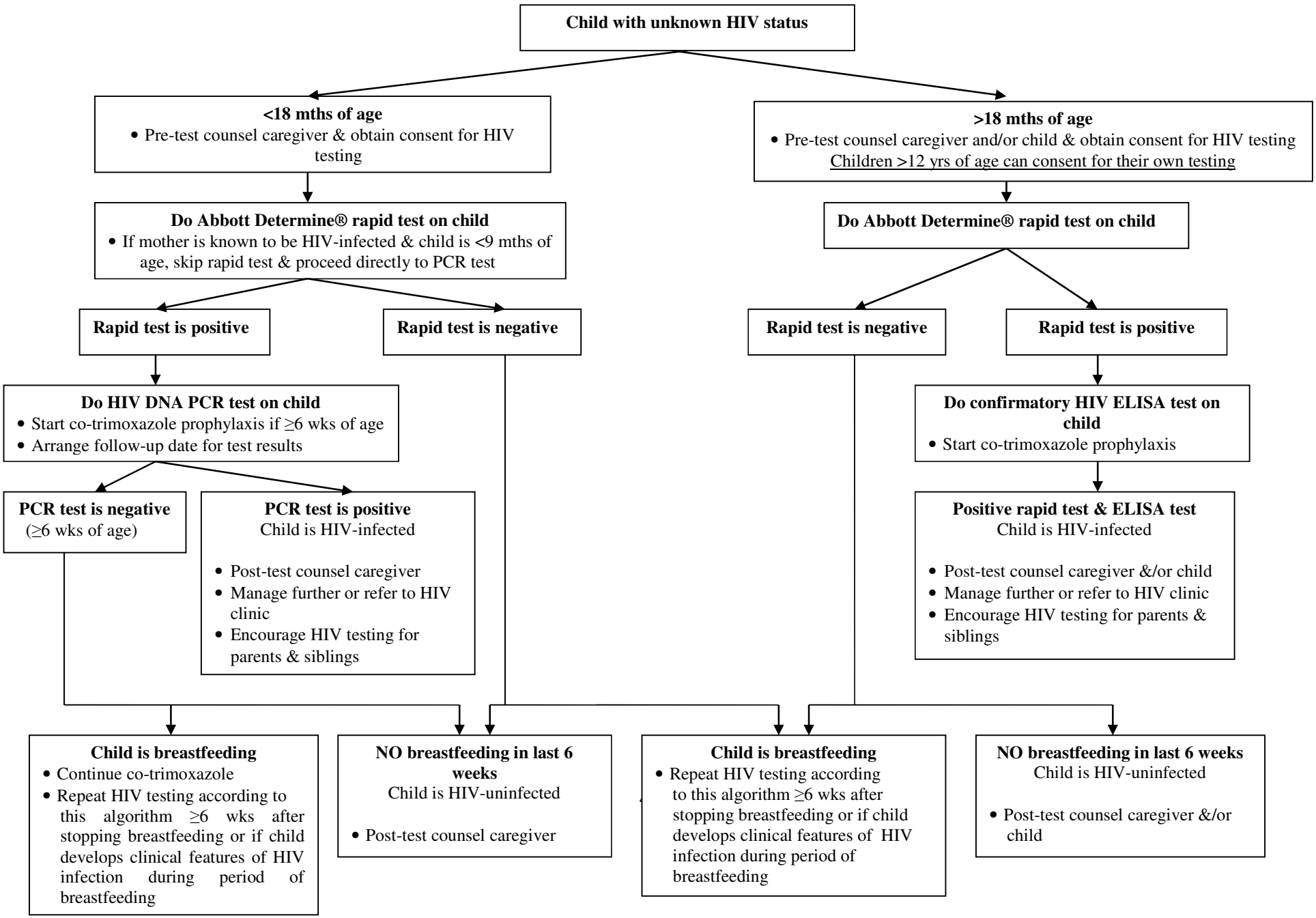


Table 6. Children undergoing HIV testing at RCWMCH in 2010

Age group	No. of HIV RT tests	HIV RT + (%)	HIV RT – (%)	Equivocal HIV RT result (%)	No. of HIV PCR tests	HIV PCR + (%)	HIV PCR – (%)	Equivocal HIV PCR result (%)
<1 mo	224	31 (13.8)	191 (85.3)	2 (0.9)	119	5 (4.2)	114 (95.8)	0
1-<2 mo	310	48 (15.5)	259 (83.6)	3 (1.0)	198	20 (10.1)	177 (89.4)	1 (0.5)
2-<3 mo	232	52 (22.4)	175 (75.4)	5 (2.2)	158	46 (29.1)	110 (69.6)	2 (1.3)
3-<4 mo	116	29 (25.0)	84 (72.4)	3 (2.6)	87	22 (25.3)	65 (74.7)	0
4-<5 mo	171	25 (14.6)	144 (84.2)	2 (1.2)	68	10 (14.7)	58 (85.3)	0
5-<6 mo	145	31 (21.4)	113 (77.9)	1 (0.7)	89	11 (12.4)	78 (87.6)	0
6-<7 mo	140	23 (16.4)	116 (82.9)	1 (0.7)	63	3 (4.8)	60 (95.2)	0
7-<8 mo	150	25 (16.7)	123 (82.0)	2 (1.3)	65	7 (10.8)	58 (89.2)	0
8-<9 mo	118	21 (17.8)	92 (78.0)	5 (4.2)	49	9 (18.4)	40 (81.6)	0
9-<10 mo	147	28 (19.1)	110 (74.8)	9 (6.1)	69	10 (14.5)	59 (85.5)	0
10-<11 mo	133	18 (13.5)	107 (80.5)	8 (6.0)	45	5 (11.1)	40 (88.9)	0
11-<12 mo	161	13 (8.1)	143 (88.8)	5 (3.1)	57	8 (14.0)	49 (86.0)	0
12-23 mo	811	41 (5.1)	748 (92.2)	22 (2.7)	130	26 (20.0)	104 (80.0)	0
24–59 mo	721	49 (6.8)	659 (91.4)	13 (1.8)	34	N/A	29 (85.3)	0
5–9 yrs	470	54 (11.5)	409 (87.0)	7 (1.5)	12	N/A	9 (75.0)	0
10–14 yrs	193	23 (11.9)	168 (87.1)	2 (1.0)	10	N/A	6 (60.0)	0
Total	4242	511	3641	90	1253	194	1056	3

The number of HIV PCR tests performed exceeds the number of positive HIV RTs as children who were known to be HIV-exposed at the time of testing frequently have an HIV PCR test without an HIV RT being performed first, RT = Rapid Test, PCR = Polymerase Chain Reaction, mo = months, yrs = years

AIM OF THE STUDY

The aim of this study was to evaluate the diagnostic accuracy of HIV RTs in use in 2011 for HIV testing in South Africa. In children <18 months of age, HIV RTs were evaluated for:

1. detection and exclusion of HIV exposure
2. detection of seroreversion
3. detection of HIV-exposed and -infected children

In children aged 18 months – <15 years, HIV RTs will be evaluated for:

4. diagnosis and exclusion of HIV infection

The standard for comparison of HIV RT results in this study wasthe Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA test and the Total Nucleic Acid (TNA) PCR test (COBAS Ampliprep/COBAS TaqMan HIV-1 Qual test (Roche).

SPECIFIC OBJECTIVES OF THE STUDY

1. To determine the sensitivity, specificity, positive and negative likelihood ratios of each of the HIV RTs in comparison to the Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA test for detection and exclusion of HIV exposure in children <18 months of age.
2. To determine the ability of the each of the HIV RTs to detect seroreversion in comparison to the Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA test in children <18 months of age
3. To determine the ability of each of the HIV RTs to detect HIV-exposed and –infected children <18 months of age in comparison to the Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA test and TNA PCR test (COBAS Ampliprep/COBAS TaqMan HIV-1 Qual test (Roche).
4. To determine the sensitivity, specificity, positive and negative likelihood ratios of each of the HIV RTs for detection and exclusion of HIV infection in comparison to the Behring Enzygnost Anti HIV1/2 Plus laboratory ELISA test in children ≥18 months - <15 years of age.

MATERIALS AND METHODS

Study design and setting

A prospective, cross-sectional laboratory study was conducted over a 3-year period between June 2011 and June 2014 in the Haematology laboratory of the NHLS at RCWMCH in Cape Town and from June 2012 – June 2014 in the Department of Molecular Medicine and Haematology, University of Witwatersrand, Johannesburg

Eligibility for inclusion in the study was the availability of leftover EDTA whole blood sample from any child <15 years of age whose blood was submitted to the laboratory for HIV testing. Blood samples that were older than 24 hours from time of arrival at the laboratory were not eligible for inclusion in the study.

Figures 2 and 3 show the study HIV testing algorithms for children <18 months of age and children ≥18 months of age respectively. The study algorithms incorporate both the routine HIV testing algorithm (Figure 1) used at RCWMCH and additional study-specific testing procedures in order to facilitate the recruitment of leftover blood sample for the study. A study HIV ELISA test was performed on all samples included in the study that did not have an HIV ELISA test performed in the routine HIV testing algorithm (including children <18 months of age with positive routine Determine RT results and children of all ages with negative routine Determine RT results) to act as the reference standard for that child against which to assess the performance of the different HIV RTs (index tests).

The 2009-2011 NDOH tender included the following HIV RTs: Advanced Quality One Step Anti HIV (1&2) (InTec Products Inc.); SD Bioline HIV 1/2 3.0 (Standard Diagnostics, Inc.); G. Ocean (additional details not available), First Response HIV 1-2.0 Card test (Premier Medical Corporation Ltd.), and Abbott Determine HIV-1/2 (Abbott Laboratories). The study was able to obtain donated stock from the NDOH of Advanced Quality, SD Bioline and Determine RTs but was unable to obtain donated or purchased stock of G. Ocean or First Response RTs. The Reveal Rapid HIV Antibody Test (MedMira Laboratories Inc.) and Insti HIV-1 RT (BioLytical

Laboratories, British Columbia, Canada) were purchased using study funding. The Insti HIV-1 RT was included in the study as it had been evaluated in 2 previous local studies and warranted further investigation due to its good performance and short testing time of 1 minute. During the course of the study, the SD Bioline RT was officially withdrawn from use in the NHLS and replaced with the Abon HIV Tri Line 1/2/0 RT (Abon Biopharm (Hangzhou) Co. Ltd.). The Abon RT was therefore included in this study following the replacement of SD Bioline.

When the amount of available EDTA whole blood sample was insufficient for both the study HIV ELISA test and the study HIV RTs to be performed, suitable leftover plasma or serum from biochemical tests submitted from the same child and taken at the same time as the sample for HIV testing was used for the study HIV ELISA test (but not for the HIV RTs) at the Cape Town study site.

Although the Determine RT is routinely performed on plasma at RCWMCH, many HIV testing facilities do not have the equipment to separate plasma and therefore for reasons of generalizability this study focussed on the performance of the Determine RT and the other HIV RTs on whole blood. The study design did not accommodate the assessment of HIV RTs performed on capillary blood samples obtained directly from finger-prick samples. Remaining whole blood sample was used for dried blood spots for later HIV TNA PCR testing if required.

At both study sites, a trained laboratory technologist with experience in HIV testing performed all study-related laboratory procedures and followed a standard operating procedure. The laboratory technologist performing the HIV RTs was blinded to the result of the HIV ELISA test and HIV PCR test at the time of performing the HIV RTs and the laboratory technologist performing the HIV ELISA or HIV PCR tests was blinded to the results of the HIV RTs. All HIV RTs were performed according to the manufacturer's instructions as stated in each package insert. [55-60] Weakly positive HIV RT results were reported as positive. Since the available volume of EDTA whole blood varied, HIV RTs were performed in the following order of priority: Insti, Reveal, SD Bioline / Abon, Advanced Quality, Determine. Depending on the volume of sample available, all or some of the HIV RTs were performed for each

patient. Sample volumes were usually inadequate to allow for routine repeating of HIV RT and/or HIV ELISA tests when results were discordant. Known negative and positive controls for HIV antibodies were obtained from the National Institute for Communicable Diseases (NICD), aliquoted and frozen. All the HIV RTs were tested at the beginning of each week with the known positive and negative controls and whenever a new batch of a particular HIV RT was used, to check the integrity of the HIV RT kits.

The study algorithms and HIV RT procedures did not interfere with the reporting of routine Determine RT, HIV ELISA or HIV PCR results to the clinician and the results of study-specific tests were not released to clinicians.

A formal sample size calculation was not undertaken prior to starting the study as it was decided that the initial study budget could accommodate a convenience sample collected over approximately one year with review of progress with respect to the rate of recruitment of samples.

The cost of the tests used in the routine HIV testing algorithm (Determine RT and HIV PCR tests for HIV-exposed children <18 months of age and HIV ELISA tests for children ≥18 months of age who test positive with Determine RT) at RCWMCH was covered by the Western Cape Province Department of Health. Funding for the remaining costs of the study including purchasing HIV RTs (some were donated by the NDOH), laboratory equipment, and employment of a laboratory technologist was from the United Children's Fund (UNICEF) and University of Witwatersrand (WITS) Health Consortium Pty Ltd. The study was not adequately funded to perform HIV PCR testing on children who tested negative with the routine Determine RT or negative with the HIV ELISA test at the RCWMCH study site.

Definitions

For the purposes of this study, the following definitions are used:

1. HIV exposure was defined as a positive laboratory HIV ELISA test (Behring Enzygnost Anti HIV1/2 Plus) result in a child <18 months of age
2. HIV infection
    a. in a child <18 months of age was defined as a positive HIV PCR test result (COBAS Ampliprep/COBAS TaqMan HIV-1 Qual test (Roche)
    b. in a child ≥18 months of age was defined as a positive Determine rapid test result followed by a positive laboratory HIV ELISA test (Behring Enzygnost Anti HIV1/2 Plus) result.
3. An equivocal laboratory HIV ELISA test was one that did not meet the laboratory criteria for a positive or negative result (the optical density is 10% above or below the cut-off value for the test).
4. An invalid laboratory HIV ELISA test or HIV rapid test was one in which no result is obtained as a result of insufficient sample volume, failure of the Control strip to appear, or failure of the blood sample or buffer to move along the test strip to reach the Test or Control lines.

Data collection

Study data comprised the date of birth of each child undergoing HIV testing, the date of blood sampling and qualitative results of all HIV RTs, HIV ELISA and HIV PCR tests. Study data was entered into an Excel spreadsheet by the study laboratory technologist using a laboratory number and study number and was accessible only to the study technologist and investigator. The data was regularly reviewed by the technologist and investigator to check for recording errors and inconsistencies.

Statistical analysis

The dataset for analysis comprises data from the Cape Town (CT) and Johannesburg (Jhb) study sites. Children with pre-defined equivocal or invalid HIV ELISA, HIV PCR or HIV RT results were excluded from the analyses and only children with a valid result (positive or negative) are included.

The laboratory HIV ELISA test results were used to calculate the prevalence of HIV exposure among children <18 months of age (by different age categories) and the prevalence of HIV infection among children ≥18 months of age. The HIV PCR test results were used to calculate the prevalence of HIV infection among children <18 months of age. The laboratory HIV ELISA test result was the reference standard for HIV exposure (in children <18 months of age) and HIV infection (in children ≥18 months of age) against which the sensitivity, specificity, and positive and negative likelihood ratios of each of the 6 HIV RTs (the index tests) are calculated.

Sensitivity (true-positive results / [true-positive + false-negative results]) refers to the proportion of children with the target condition (HIV exposure for children <18 months of age or HIV infection for children ≥18 months of age) who tested positive with the index test (HIV RT). Specificity (true-negative / [true-negative + false-positive results]) refers to the proportion of children without the target condition (HIV exposure for children <18 months of age or HIV infection for children ≥18 months of age) who tested negative with the index test (HIV RT). Sensitivity and specificity values across different age categories for each of the 6 HIV RTs were calculated with 95% CI using the Wilson score method (Confidence interval calculator, Physiotherapy Evidence Database (PED), version 24 March 2011, available at www.pedro.org.au/wp-content/uploads/CIcalculator.xls).

Since the prevalence of HIV exposure and infection varied across each age category, calculation of positive and negative predictive values was precluded. Positive and negative likelihood ratios (LR+ and LR-) were used instead. The likelihood ratio of a positive test provides a measure of how much more likely a positive test result is in someone with the target condition as compared with someone without the target

condition. A LR+ >1 indicates an increased probability that the target condition is present and an LR- <1 indicates a decreased probability that the target condition is present. LR+ and LR- values of >10 and <0.1 respectively indicate a very high or very low likelihood of the target condition being present. In this study, a positive likelihood ratio of >10 strongly predicted HIV exposure (in the analysis of HIV RT in comparison to laboratory HIV ELISA for detection of HIV exposure in children <18 months of age) or HIV infection (in the analysis of HIV RT in comparison to HIV ELISA for diagnosis of HIV infection in children ≥18 months of age). Similarly, a negative likelihood ratio of <0.1 strongly predicted exclusion of HIV exposure or infection. [61]

The laboratory HIV ELISA and HIV PCR test results were also used to calculate seroreversion rates for the different HIV RTs across different age categories and the ability of each of the HIV RTs to detect HIV-exposed and infected children <18 months of age.

Ethics considerations

The study protocol was approved by the Departmental Research Committee of the Department of Paediatrics and Child Health, University of Cape Town and the Human Research Ethics Committee of the University of Cape Town (HREC REF 218/2011). Permission to conduct the study at RCWMCH was obtained from the hospital research committee. The Johannesburg study site obtained approval to conduct the study from the University of the Witwatersrand Ethics Committee (Protocol approval M09-06-88).

Figure 2. HIV testing algorithm for children <18 months of age (incorporating routine laboratory and study technologist roles)

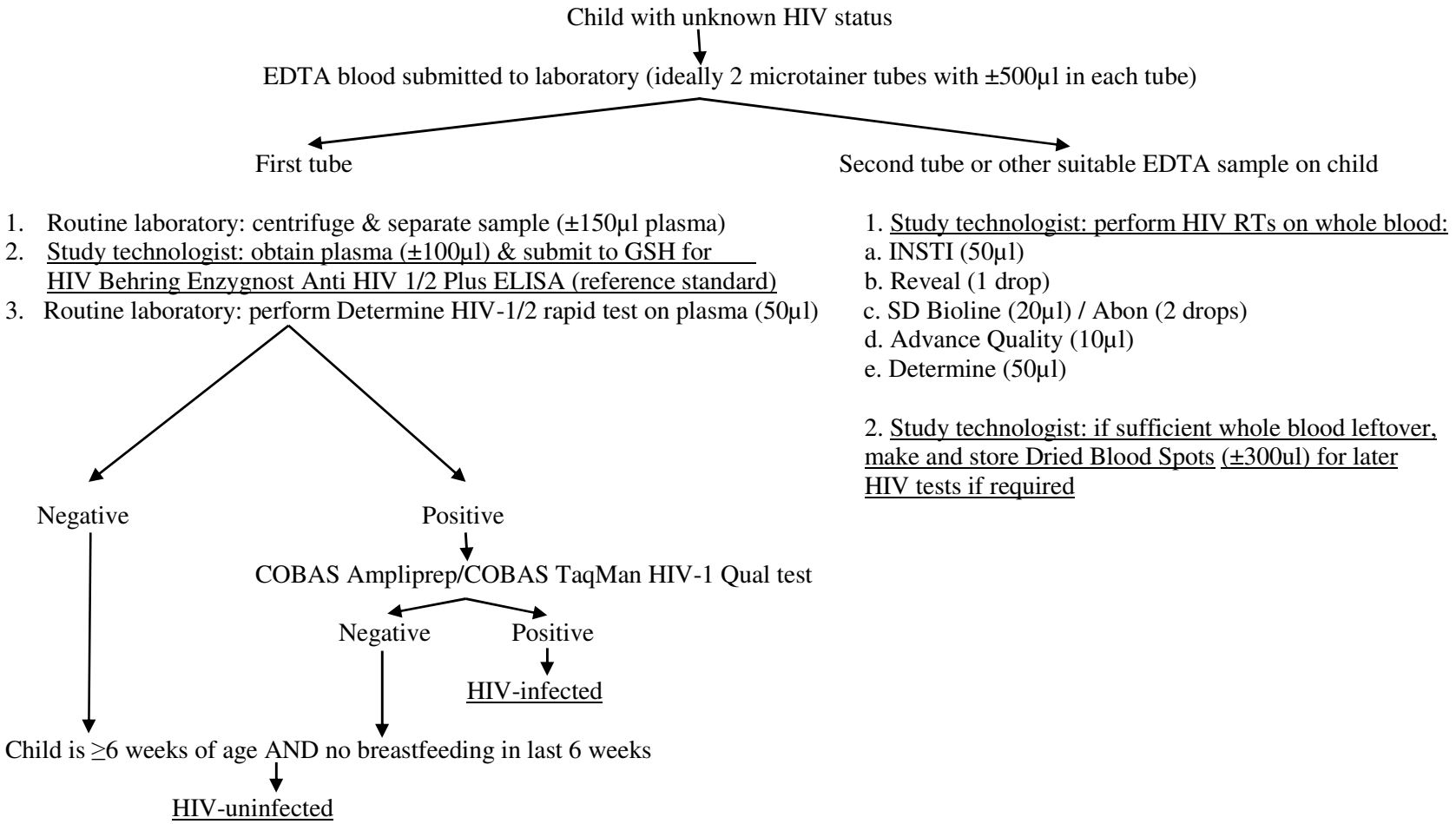
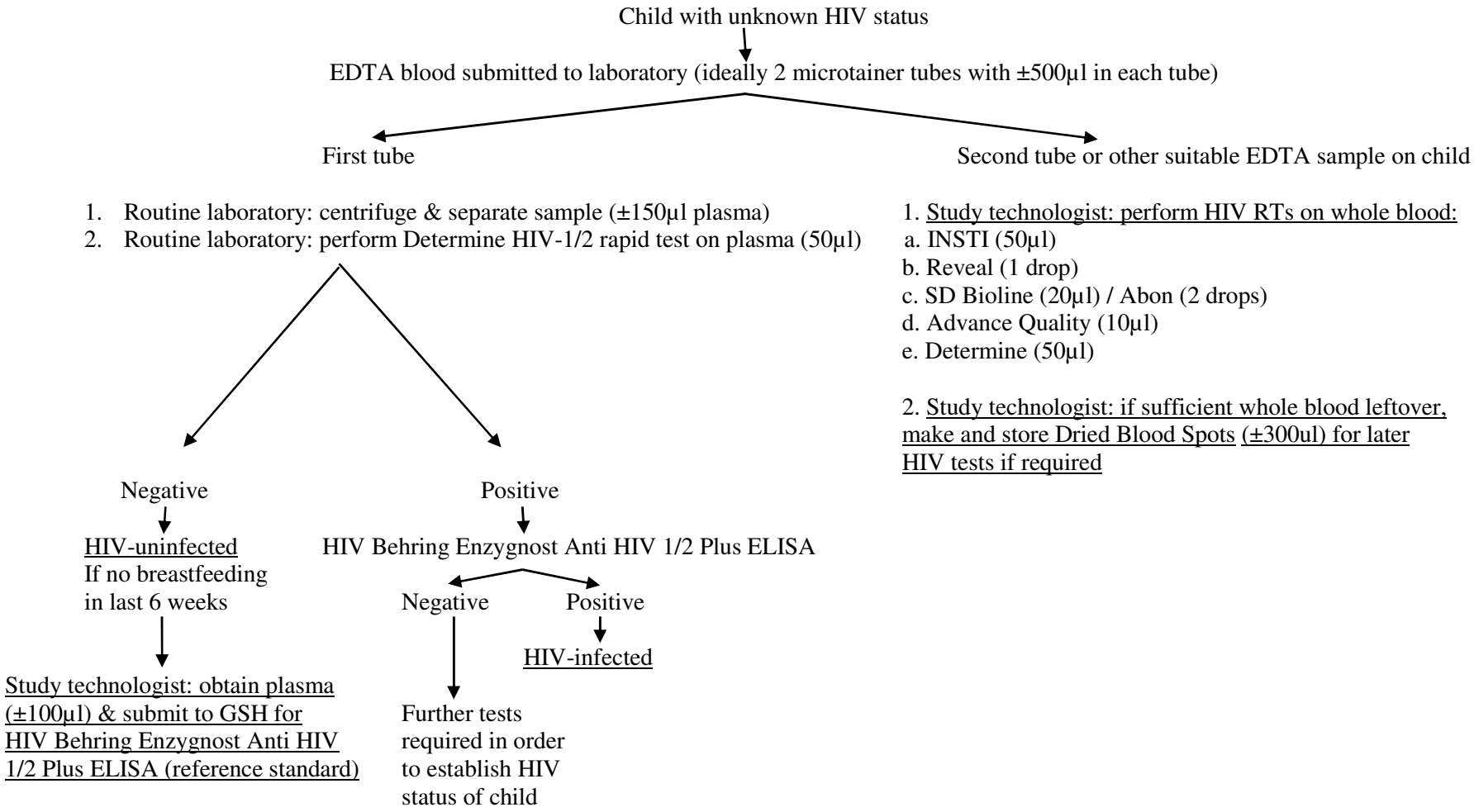


Figure 3. Study HIV testing algorithm for children ≥18 months of age (incorporating routine laboratory and study technologist roles)



RESULTS

The flow of study participants in relation to the study tests performed is shown in Figure 4. A total of 1173 children <15 years of age had laboratory HIV ELISA testing done, 754 at the CT study site and 419 at the Jhb study site. There were 4 (0.3%) children (all <9 months of age) with an equivocal HIV ELISA test result (all at the CT study site), and 10 (0.9%) children (all at the Jhb study site) in whom the HIV ELISA test was invalid. The 14 children with equivocal or invalid HIV ELISA tests were excluded from the statistical analysis. In these 14 children, the results of the HIV RTs varied but all had a negative HIV PCR test. The study population with a valid HIV ELISA test result included in the statistical analysis comprised 1159 children of which 896 were <18 months of age (Table 7) and 263 were ≥18 months of age (Table 8). Among children with a valid HIV ELISA result, the highest number of tests (353/896, 39.4%) was performed in children <3 months of age and the lowest number (54/896, 6.0%) in children 15-<18 months of age. Among the 896 children <18 months of age with a valid HIV ELISA result, a positive result was obtained in 488/896 giving an overall HIV exposure rate of 54.5% ranging from 69.1% (125/181) in the 3-<6 months age group to 13.2% (10/76) in the 12-<15 months age group. Among the 263 children ≥18 months of age with a valid HIV ELISA result (262 from the CT site and 1 from the Jhb site) the HIV prevalence rate was 4.9% (13/263) and there were no equivocal results or invalid HIV ELISA tests.

A total of 5768 HIV RTs were performed on whole blood, 3673 at the CT study site and 2095 at the Jhb study site. There were 32 HIV RTs (31 Reveal and 1 Abon RT) with invalid results (all at the Jhb study site) and these were excluded from the statistical analysis. A total of 5736 HIV RTs were included in the statistical analysis. Among the 896 children <18 months of age with a valid HIV ELISA result, a total of 4446 valid HIV RT results were obtained ranging from 1752 HIV RTs in the <3 month age group to 266 HIV RTs in the 15-<18 month age group (Table 9). Among the 263 children ≥18 months of age with a valid HIV ELISA result, a total of 1290 HIV RTs were performed (Table 10). The wide variation in the number of children undergoing testing with each of the RTs is because the volume of available blood sample for each child varied and because HIV RTs were performed in a protocol-

defined sequence of priority. In addition, during the course of the study, the SD Bioline RT was withdrawn from use in the Department of Health and was replaced with the Abon RT.

Valid HIV PCR test results were available for 78.7% (705/896) of the children <18 months of age, 298 from the CT study site and 408 from the Jhb study site. One child (CT study site) tested positive with the HIV ELISA test and all the HIV RTs but the HIV PCR test result was equivocal. This child was excluded from the analysis of HIV RT performance in relation to detecting HIV PCR positive children. The HIV PCR test was repeated 2 days later (outside of the study) and the result was equivocal again. Unfortunately, the child was subsequently lost to follow-up. There was one child in the 15-<18-month age category (Jhb site) in whom the HIV ELISA test was negative but all the HIV RTs (except Reveal) were positive and the HIV PCR test was positive. There was insufficient sample available to repeat the tests and this child was excluded from the analysis of HIV RT performance for detection of HIV exposure and HIV infection in view of the discordant results. Among children <18 months of age with both a valid HIV ELISA and a valid HIV PCR result, the overall HIV prevalence was 9.6% (68/705) ranging from 41.9% (13/31) in the 15-<18 month age group to 5.7% (17/299) in the <3 month age group (Table 7).

Children <18 months of age

Diagnostic performance of HIV RTs for detection and exclusion of HIV exposure

The sensitivity (95% CI) and positive likelihood ratio (LR+) of the 6 HIV RTs for detection of HIV exposure (HIV ELISA+) in children <18 months of age is shown in Table 11. The overall sensitivity values ranged from 38.7% (Reveal) to 94.7% (Determine). In children <3 months of age, the sensitivity values ranged from 56.3% (Reveal) to 98.7% (Determine) with all HIV RTs except Reveal detecting between 94.9 – 98.7% of HIV-exposed infants. Positive likelihood ratios were all >10 indicating strong prediction of correctly identifying HIV exposure. Positive likelihood ratios could not be calculated for 3 HIV RTs (Reveal, Abon, SD Bioline) that had 100% specificity for detection of HIV exposure (no false-positive results).

In a subset of children at the CT study site, the Determine RT was also performed on plasma in the routine HIV testing laboratory (Figures 2 and 3). The sensitivity of the Determine RT performed on plasma in children <18 months of age was higher than the sensitivity on whole blood (98.7 versus 94.7%) but the 95% CIs were overlapping (Table 11). In children <3 months of age, the sensitivity of the Determine RT was similar on plasma compared with whole blood (96.6 versus 98.7%).

The specificity (95% CI) and negative likelihood ratio (LR-) of each of the 6 HIV RTs for excluding HIV exposure was assessed in the 408 HIV ELISA negative children <18 months of age and is shown in Table 12. The Insti, Reveal, SD Bioline and Advanced Quality RTs all showed specificities >98% with 3, 0, 1, and 2 false positive results respectively. Six Abon and 27 Determine RT false-positive results were obtained with specificities of 97.0% (95% CI 93.6-98.6) and 93.1% (95% CI 90.1-95.2) respectively but both these HIV RTs had negative likelihood ratios of 0.1. In children <3 months of age, the specificities of all the HIV RTs were >98% except Determine for which 3 false positive results were obtained with a specificity of 97.4% (95% CI 92.6-99.1).

The specificity of the Determine RT performed on plasma in a subset of children at the CT study site was similar to that on whole blood for children <18 months of age and children <3 months of age (92.6 versus 93.1% and 98.1 versus 97.4% respectively) as shown in Table 12.

Diagnostic performance of HIV RTs for detection of seroreversion

The performance of the 6 HIV RTs in detecting seroreversion was assessed as the percentage of negative HIV RT results in HIV-exposed and uninfected (HIV ELISA+, HIV PCR-) children <18 months of age (Table 13). Among infants <4 months of age, 5 of the 6 HIV RTs had seroreversion rates of <15%. The Reveal HIV RT showed seroreversion rates of up to 64% in infants aged between 2 and 4 months of age but this is likely to be related to the low sensitivity of this RT.

Although much higher rates of seroreversion were detected among children >8 months of age for most of the HIV RTs, a relatively low number of samples in children between 8 and 18 months of age limited the value of this analysis. The Determine RT showed the lowest rate of seroreversion in comparison to the other HIV RTs across all the age categories in keeping with the high sensitivity of this RT.

Diagnostic performance of HIV RTs for detection of HIV exposed and –infected children

The performance of the 6 HIV RTs in detecting HIV-infected (HIV PCR+) children is shown in Table 14. All of the HIV RTs except the Reveal RT detected 95-100% of HIV-infected children with the Abon, Advanced Quality and Determine RTs detecting 100% of HIV-infected children <18 months of age. The Reveal RT missed 27/65 (41.5%) HIV-infected children mostly <8 months of age and 16-18 months of age. The SD Bioline RT missed 1 child of 11-12 months of age out of 22 HIV-infected children (4.5%) and the Insti RT missed 1 child of 3-4 months of age out of 68 HIV-infected children (1.5%).

In the 8-10 month age category during which time the WHO recommends routine screening for HIV infection using HIV RTs, no HIV-infected children were missed by any of the HIV RTs however the number of HIV-infected children (HIV PCR+) was very low. Under 3 months of age, only the Reveal RT was unable to detect 100% of HIV-infected children.

Children ≥18 months of age

Diagnostic performance of HIV RTs for detection and exclusion of HIV infection

The sensitivity values ranged from 69.2% (9/13) with the Reveal RT to 100% with the Abon (4/4), SD Bioline (8/8) and Determine (13/13) RTs respectively (Table 15). Confidence intervals were wide owing to the small number of HIV ELISA positive children ≥18 months of age. The specificity of the 6 HIV RTs for excluding HIV infection was 100% (Table 16). The positive and negative likelihood ratios indicated

that these 6 HIV RTs were strongly able to predict or exclude HIV infection in this age category.

Figure 4. Flow of study participants in relation to study tests performed

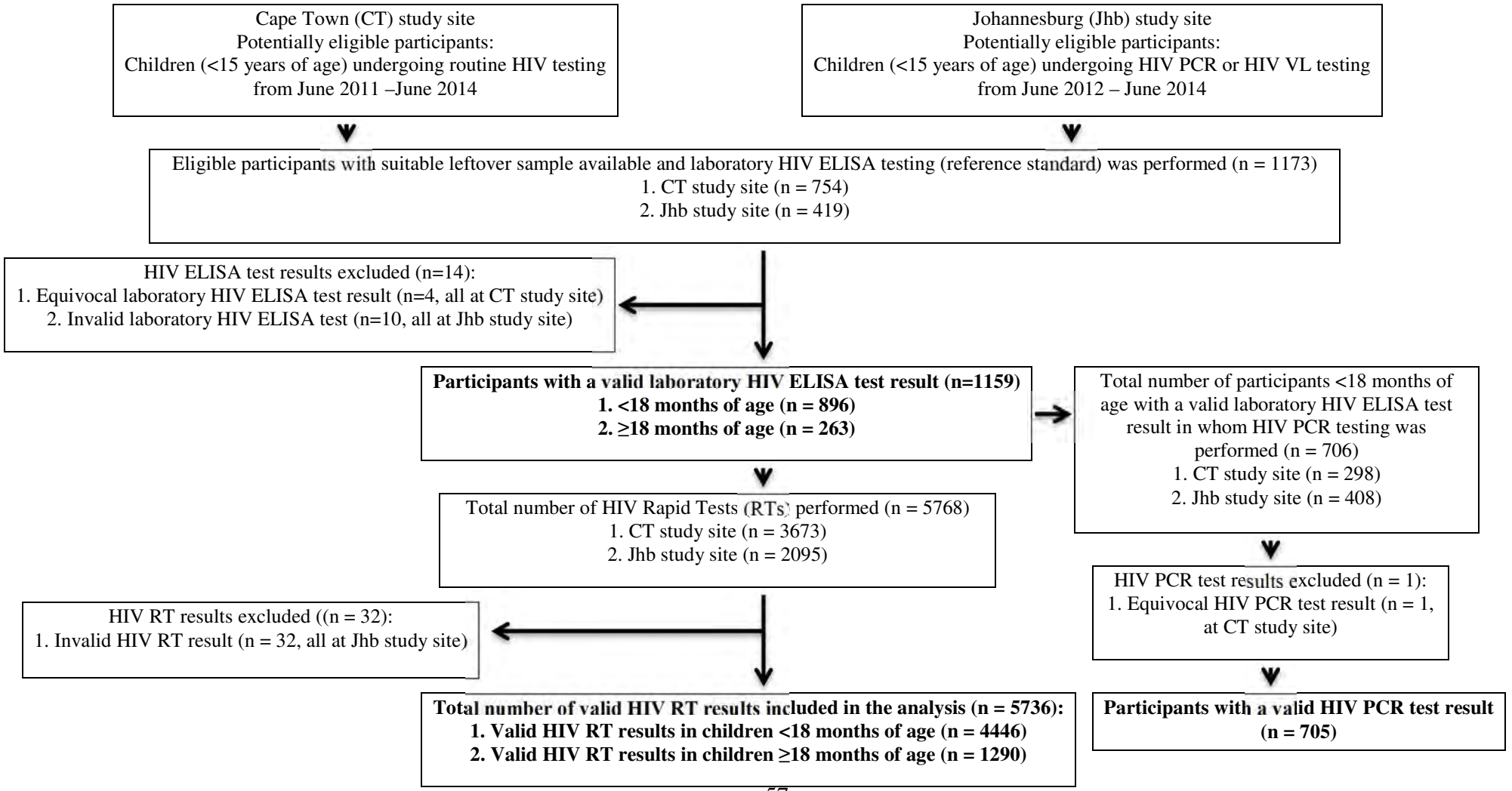


Table 7. Description of study cohort: children <18 months of age

Age (mo)	Valid HIV ELISA results	HIV ELISA+ (HIV exposed)	HIV ELISA- (HIV unexposed)	HIV exposure HIV ELISA+ / (HIV ELISA+ plus HIV ELISA-) (%)	Equivocal HIV ELISA results	Failed HIV ELISA tests	Valid HIV PCR results	HIV PCR+ (HIV infected)	HIV PCR- (HIV uninfected)	HIV prevalence HIV PCR+ / (HIV PCR+ plus HIV PCR-) (%)	Equivocal HIV PCR results	No HIV PCR result available
0-<3	353	234	119	234/353 (66.3)	1	3	299	17	281	17/299 (5.7)	1	54
3-<6	181	125	56	125/181 (69.1)	2	5	154	17	135	17/154 (11.0)	0	29
6-<9	142	83	59	83/142 (58.5)	1	0	116	11	104	11/116 (9.5)	0	27
9-<12	90	24	66	24/90 (26.7)	0	1	59	7	52	7/59 (11.9)	0	31
12-<15	76	10	66	10/76 (13.2)	0	0	50	3	47	3/50 (6.0)	0	26
15-<18	54	12	42	12/54 (22.2)	0	1	31	13	18	13/31 (41.9)	0	23
Total	896	488	408	488/896 (54.5)	4	10	705	68	637	68/705 (9.6)	1	190

mo = months, ELISA = Enzyme Linked Immunosorbent Assay, PCR = Polymerase Chain Reaction

Table 8. Description of study cohort: children ≥18 months of age

Age (mo)	Total HIV ELISA tests	HIV ELISA+ (HIV infected)	HIV ELISA- (HIV uninfected)	Equivocal HIV ELISA result	HIV prevalence HIV ELISA+ / (HIV ELISA+ plus HIV ELISA-) (%)
≥18	263	13	250	0	13/263 (4.9)

mo = months, ELISA = Enzyme Linked Immunosorbent Assay

Table 9. Spectrum of HIV rapid tests (RTs) performed in children <18 months of age (by age group)

Study cohort		Valid HIV RT results						
Age (mo)	**Valid HIV ELISA results**	**Insti**	**Reveal**	**Abon**	**SD Bioline**	**Advanced Quality**	**Determine**	**Total HIV RTs**
<3	353	353	336	203	179	342	339	1752
≥3-<6	181	181	175	108	86	178	173	901
≥6-<9	142	142	138	91	57	141	138	707
≥9-<12	90	90	90	45	50	89	87	451
≥12-<15	76	76	72	46	35	71	69	369
≥15-<18	54	54	53	26	25	54	54	266
Total <18	896	896	864	519	432	875	860	4446

mo = months, ELISA = Enzyme Linked Immunosorbent Assay

Table 10. Spectrum of HIV rapid tests (RTs) performed in children ≥18 months of age

Study cohort		HIV RTs						
Age (mo)	**Valid HIV ELISA tests**	**Insti**	**Reveal**	**Abon**	**SD Bioline**	**Advanced Quality**	**Determine**	**Total HIV RTs**
≥18	263	263	263	24	215	263	262	1290

mo = months, ELISA = Enzyme Linked Immunosorbent Assay

Table 11. Sensitivity (95% confidence interval [CI]) and positive likelihood ratio (LR+) of each HIV rapid test (HIV RT) for detection of HIV exposure (HIV ELISA+) in children <18 months and <3 months of age

HIV RT	Total HIV ELISA+	HIV ELISA+ HIV RT+	Sensitivity (%)	95% CI	LR+
<18 mo of age					
Insti	488	355	72.8	68.6-76.5	98.9
Reveal	468	181	38.7	34.4-43.2	-
Abon	319	275	86.2	82.0-89.6	28.7
SD Bioline	202	171	84.7	79.0-89.0	194.7
Advanced Quality	475	379	79.8	76.0-83.2	159.6
Determine (whole blood)	469	444	94.7	92.3-96.4	13.7
Determine (plasma)#	75	74	98.7	92.8-99.8	13.4
<3 mo of age					
Insti	234	222	94.9	91.3-97.0	112.9
Reveal	222	125	56.3	49.7-62.7	-
Abon	137	134	97.8	93.8-99.3	-
SD Bioline	110	107	97.3	92.3-99.1	-
Advanced Quality	226	222	98.2	95.5-99.3	113.9
Determine (whole blood)	225	222	98.7	96.2-99.6	37.5
Determine (plasma)#	29	28	96.6	82.8-99.4	51.2

mo = months, ELISA = Enzyme Linked Immunosorbent Assay
# Determine RT on plasma performed in routine HIV testing laboratory at RCWMCH

Table 12. Specificity (95% confidence interval [CI]) and negative likelihood ratio (LR-) of each HIV rapid test (HIV RT) for excluding HIV exposure (HIV ELISA-) in children <18 months of age

HIV RT	Total HIV ELISA-	HIV ELISA- HIV RT+	Specificity	95% CI	LR-
<18 mo of age					
Insti	408	3	99.3	97.9-99.8	0.3
Reveal	396	0	100	99.0-100	0.6
Abon	200	6	97.0	93.6-98.6	0.1
SD Bioline	230	1	99.6	97.6-99.9	0.2
Advanced Quality	400	2	99.5	98.2-99.9	0.2
Determine (whole blood)	391	27	93.1	90.1-95.2	0.1
M Determine (plasma)#	190	14	92.6	88.0-95.6	0
<3 mo of age					
Insti	119	1	99.2	95.4-99.9	0.1
Reveal	114	0	100	96.7-100	0.4
Abon	66	0	100	94.5-100	0
SD Bioline	69	0	100	94.7-100	0
Advanced Quality	116	1	99.1	95.3-99.9	0
Determine (whole blood)	114	3	97.4	92.6-99.1	0
Determine (plasma)#	53	1	98.1	90.1-99.7	0

mo = months, ELISA = Enzyme Linked Immunosorbent Assay
# Determine RT on plasma performed in routine HIV testing laboratory at RCWMCH

Table 13. Performance of each HIV rapid test (HIV RT) in detecting seroreversion (SR) in HIV-exposed and –uninfected (HIV ELISA+ HIV PCR-) children <18 months of age

Age (mo)	Insti		Reveal		Abon		SD Bioline		Advanced Quality		Determine	
	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR	HIV RT-/ (HIV ELISA+ HIV PCR-)	%SR
<2	6/158	3.8	57/148	38.5	3/101	3.0	3/73	4.1	4/154	2.6	3/153	2.0
2-<4	14/100	14.0	62/96	64.6	1/61	1.6	0/46	0	4/95	4.2	0/93	0
4-<6	35/61	57.4	53/60	88.3	1/39	2.6	5/28	17.9	15/61	24.6	0/60	0
6-<8	45/58	77.6	54/57	94.7	16/41	39.0	11/20	55.0	44/58	75.9	6/56	10.7
8-<10	17/17	100	15/16	93.8	11/13	84.6	7/7	100	16/17	94.1	7/17	41.2
10-<12	8/9	88.9	8/9	88.9	8/9	88.9	3/3	100	7/8	87.5	6/8	75.0
12-<18	3/6	50.0	5/6	83.3	3/6	50.0	6/6	100	3/6	50.0	3/6	50.0

mo = months, ELISA = Enzyme Linked Immunosorbent Assay, PCR = Polymerase Chain Reaction

Table 14. Performance of each HIV rapid test (HIV RT) in detecting HIV-infected (HIV PCR+) children <18 months of age (HIV PCR+, HIV RT+)

HIV RT	HIV PCR+	HIV PCR+ HIV RT+	HIV PCR+ HIV RT-	% HIV PCR+ detected by HIV RT alone
<18 mo of age				
Insti	68	67	1	98.5
Reveal	65	38	27	58.5
Abon	41	41	0	100
SD Bioline	22	21	1	95.5
Advanced Quality	65	65	0	100
Determine	65	65	0	100
8-10 mo of age				
Insti	3	3	0	100
Reveal	3	3	0	100
Abon	1	1	0	100
SD Bioline	2	2	0	100
Advanced Quality	3	3	0	100
Determine	3	3	0	100
<3 mo of age				
Insti	17	17	0	100
Reveal	16	10	6	62.5
Abon	6	6	0	100
SD Bioline	7	7	0	100
Advanced Quality	16	16	0	100
Determine	16	16	0	100

mo = months, PCR = Polymerase Chain Reaction

Table 15. Sensitivity (95% confidence interval [CI]) and positive likelihood ratio (LR+) of each HIV rapid test (HIV RT) for diagnosis of HIV infection (HIV ELISA+) among children ≥18 months of age

HIV RT	Total HIV ELISA+	HIV ELISA+ HIV RT+	Sensitivity	95% CI	LR+
Insti	13	12	92.3	66.7-98.6	92.3
Reveal	13	9	69.2	42.4-87.3	69.2
Abon	4	4	100	51.0-100	100
SD Bioline	8	8	100	67.6-100	100
Advanced Quality	13	12	92.3	66.7-98.6	92.3
Determine	13	13	100	77.2-100	100

ELISA = Enzyme Linked Immunosorbent Assay

Table 16. Specificity (95% confidence interval [CI]) and negative likelihood ration (LR-) of each HIV rapid test (HIV RT) for excluding HIV infection (HIV ELISA-) in children ≥18 months of age

HIV RT	Total HIV ELISA-	HIV ELISA- HIV RT+	Specificity	95% CI	LR-
Insti	250	0	100	98.5-100	0.1
Reveal	250	0	100	98.5-100	0.1
Abon	20	0	100	83.9-100	0
SD Bioline	207	0	100	98.2-100	0
Advanced Quality	250	0	100	98.5-100	0.1
Determine	249	0	100	98.5-100	0

ELISA = Enzyme Linked Immunosorbent Assay

DISCUSSION

The WHO recommendations on the diagnosis of HIV infection in infants and children incorporate the use of HIV antibody detection assays for two main roles. Firstly, as a screening assay to determine HIV exposure in children <18 months of age, and secondly, for the diagnosis of HIV infection in children ≥18 months of age. The 2010 WHO guidelines recommend HIV antibody screening of infants with uncertain HIV exposure at or around birth, at the first postnatal visit (4-6 weeks of age) or other child health visit, at around 9 months of age, and in infants with signs or symptoms suggestive of HIV infection. For children ≥18 months of age with suspected HIV infection or HIV exposure, the guidelines strongly recommend that HIV antibody testing should be performed according to the standard diagnostic HIV testing algorithm used in adults. The guidelines also make provision for using HIV antibody detection assays in combination with a clinical algorithm for presumptive diagnosis of HIV infection in children <18 months of age in settings where testing with HIV viral detection assays is not available. [9]

These guidelines strongly recommended that HIV antibody detection assays used for clinical diagnostic testing (either screening or diagnostic testing) should have a minimum sensitivity of 99% and specificity of 98% under quality-assured, standardised and validated laboratory conditions but acknowledged only moderate quality of evidence available to support this recommendation. [9] Recent WHO HIV testing guidelines (2015) emphasise the important role of virological testing in HIV-exposed infants from 4-6 weeks of age in order to rapidly identify HIV-infected infants who can start cART and also highlight that a negative HIV antibody test (including rapid tests) in an infant does not completely rule out HIV exposure and infection, especially between 4-18 months of age as a result of reduced sensitivity during seroconversion when HIV infection is acquired postpartum through breastfeeding. As with the 2010 guidelines, HIV antibody testing is still strongly recommended at 9 months of age for HIV exposed infants whose virological tests are negative at 4-6 weeks of age although the quality of evidence for this recommendation is acknowledged as being low. [62]

This study evaluated the diagnostic accuracy of 6 HIV RTs in children <15 years of age using whole blood. In order to maximize the completeness and transparency of information provided in reporting this study, the Standards for Reporting Diagnostic Accuracy (STARD) 2015 checklist of 30 essential items has been incorporated (Appendix 4). [63] This appendix shows that a concerted effort was made to include as many of the STARD checklist items as possible into the study design, analysis, and structure and content of this report.

Children <18 months of age

None of the 6 HIV RTs evaluated achieved an overall sensitivity of ≥99% in comparison to laboratory HIV ELISA testing for the detection of HIV exposure among all children <18 months of age. The Determine RT was closest to attaining the WHO standard with overall sensitivity of 94.7% (95% CI: 92.3-96.4) for children <18 months of age but failed to detect 25 HIV-exposed children, including 3 HIV-exposed children <3 months of age. In children <3 months of age, the WHO standard was almost attained by the Determine RT with a sensitivity of 98.7% (95% CI 96.2-99.6) and a positive likelihood ratio of 37.5. Amongst the other HIV RTs, Reveal was an outlier with very low sensitivity of 38.7% (95% CI 34.4-43.2) <18 months of age and 56.3% (95% CI 49.7-62.7) <3 months of age whereas the sensitivity of Insti, Abon, SD Bioline and Advanced Quality RTs ranged between 72.8-86.2% and 94.9-98.2% for children <18 months of age and children <3 months of age respectively. In a subset of 75 children <18 months of age who underwent testing with the Determine RT on plasma in the routine HIV testing laboratory at the CT study site, the sensitivity for detection of HIV exposure (HIV ELISA+) was 98.7% (95% CI 92.8-99.8). This is in keeping with other studies that have shown a higher sensitivity of screening with HIV RTs when using plasma compared to whole blood. [42,43] However, outside of laboratory settings where centrifuge facilities are available, most HIV testing makes use of whole blood from venesection or capillary blood samples.

The sensitivity of antibody screening in children <18 months of age reduces with age as the rate of seroreversion increases. In this study, the sensitivity of the HIV RTs for

detection of HIV exposure (HIV ELISA+) in children aged 4-18 months ranged between 20.7% (Reveal RT) and 88.5% (Determine RT). Between 6 and 9 months of age, all the HIV RTs had sensitivity <65% except the Determine RT (88.8% [95% CI 80.0-94.0]). Between 9 and 12 months of age, all 6 HIV RTs had sensitivity for detection of HIV exposure <60% but confidence intervals were wide due to relatively low numbers of children undergoing testing in this age group. By 15-18 months of age all the HIV RTs except Reveal (45.5% [95% CI 21.3-72.0]) had sensitivity of 100% although with wide confidence intervals due to low numbers. By far the majority of children in the 15-18 month age category were true positive (HIV RT+, HIV PCR +) or true negative (HIV RT-, HIV PCR-) results but there were 6 false negative (HIV RT-, HIV PCR+) results (all Reveal) and 7 false positive results (Insti, Abon, Advanced Quality each 1, and Determine 4).

In the evaluation of specificity of the HIV RTs for exclusion of HIV exposure, assessed in the HIV-unexposed (HIV ELISA-) children <18 months of age, the Insti, Reveal, SD Bioline and Advanced Quality RTs all achieved the WHO-recommended minimum specificity of 98%. The specificity of the Determine RT was 93.1% (95% CI 90.1-95.2) and the Abon RT was 93.6% (95% CI 93.6-98.6). In children <3 months of age only the Determine RT had specificity of <98% (97.4% [95% CI 92.6-99.1]). The implication of these findings is that using the Determine or Abon RTs to screen for HIV exposure in children <18 months of age on whole blood would detect a greater number of false positive (HIV RT+, HIV ELISA-) results than with the other HIV RTs. This would necessitate more HIV PCR tests in order to exclude HIV infection than with the other HIV RTs. In the subset of 75 children <18 months of age in whom the Determine RT was performed on plasma, the specificity was >98%.

The performance of HIV RTs in detecting seroreversion while at the same time not missing any HIV-infected children is important in the decision of how best to use HIV RTs for excluding HIV infection among HIV-exposed children <18 months of age. Screening of HIV exposed infants at around 9 months of age with an HIV antibody test, as recommended by WHO, provides 2 important public health opportunities. Firstly, if the HIV antibody test result on the infant is positive, a

virological test is required to identify HIV-infected infants who need cART. Secondly, the use of a sensitive and specific HIV RT at around 9 months of age could exclude HIV infection among well HIV-exposed infants who have not breastfed in the previous 6 weeks and provide a same-day result to the infant's caregiver without the need for expensive HIV PCR testing which usually requires the caregiver to return to the healthcare facility days to weeks later for the result.

This study showed seroreversion rates among HIV-exposed and uninfected (HIV ELISA+, HIV PCR-) children at 8-10 months of age of >80% for all the HIV RTs other than Determine (41.2%). By 10-12 months of age, the seroreversion rate was ≥75% for all the HIV RTs. There were 6 HIV PCR+ children in the 10-12 month age category that were tested with the Insti, Reveal, Advanced Quality and Determine RTs, 5 that were tested with the Abon RT and 1 tested with the SD Bioline RT. Other than the Reveal RT that missed one HIV-infected child, all the other HIV RTs detected all the HIV-infected children in the 10-12 month age category. These findings indicate that it would have been possible to accurately exclude HIV infection by screening with the Insti, Abon, SD Bioline, Advanced Quality or Determine RTs without the need for HIV PCR testing in at least 75% of the infants aged 10-12 months although breastfeeding infants would still require further testing after weaning. Unfortunately the low numbers of HIV-exposed and uninfected children in the 8-18 month age categories (32) and HIV PCR + children in the 10-12 month age category (6) limit the statistical certainty of these findings.

Overall, 3 of the HIV RTs missed HIV-infected children (HIV PCR+, HIV RT-) <18 months of age: Reveal (27/65, 41.5%), SD Bioline (1/22, 4.5%), and Insti (1/68, 1.5%). In children <3 months of age, the Reveal RT missed 6/16 (37.5%) HIV-infected children and the other HIV RTs detected 100% of HIV-infected children.

Although meeting neither the WHO sensitivity nor the specificity criteria for the <18 months age group as a whole, the Determine RT showed the best overall performance of the 6 HIV RTs evaluated. It showed the highest sensitivity for detecting HIV exposure and did not miss any HIV-infected children. Due to its high sensitivity, the Determine RT is able to detect lower amounts of circulating maternal

antibody in the infant plasma for longer than most other HIV RTs and therefore, when using the Determine RT to exclude HIV infection in HIV-exposed children <18 months of age, seroreversion (loss of maternal antibody) may be detected at a later age than with other HIV RTs as antibody levels wane to even lower levels. Therefore, false-positive results (in comparison to laboratory HIV ELISA testing) may occur and HIV PCR testing may be more frequently required to exclude HIV infection than with other HIV RTs.

Children ≥18 months of age

In children ≥18 months of age, the WHO recommended sensitivity of ≥99% in comparison to laboratory HIV ELISA for diagnosis of HIV infection was attained for 3 of the HIV RTs (Abon, SD Bioline and Determine all 100%) although confidence intervals were wide due to the small numbers of HIV-infected children in this age category. All the HIV RTs met the WHO specificity criterion and were found to have a specificity of 100% for excluding the diagnosis of HIV infection. The implication of these findings is that in a serial testing algorithm for the diagnosis of HIV infection in children ≥18 months of age, any of the 3 more sensitive HIV RTs (Abon, SD Bioline and Determine) can be recommended as the initial test, and any of the HIV RTs (which all showed high specificity for HIV infection) can be recommended as the confirmatory test (with the exception of the Reveal RT due to its very low sensitivity).

Comparison with published studies

This study confirms the finding of previous studies that all HIV RTs do not perform equally in infants and young children and contributes new data on HIV RTs not previously evaluated in very young children. There is a lack of published literature on the performance of the Reveal, Abon, SD Bioline and Advanced Quality HIV RTs in an infant or paediatric population against which to compare the findings of the present study. However, Sherman evaluated 2 of the HIV RTs included in this study, Determine and Insti, in 2 previous studies.

In the first Sherman study (2008), the sensitivity of the Determine and Insti RTs for the detection of HIV exposure in children <12 months of age was 99.7% (95% CI 99.0-100.3) and 79.9% (95% CI 75.5-84.2) respectively, slightly higher than the sensitivity of 95.1% (95% CI 92.7-96.7) and 72.1% (95% CI 67.9-76.0) respectively in children <12 months of age in the present study. [43] One of the reasons for this difference may be that in the Sherman study HIV RTs were performed on samples from HIV-exposed infants taken at 4 time points (1.5, 3, 7 and 12 months) as compared to testing across all ages in children <12 months in the present study. Another reason may be that in the Sherman study, HIV RTs were performed on stored plasma or serum whereas in the present study testing was performed on whole blood. The sensitivity of HIV RT's has been reported to be higher when performed on plasma or serum rather than whole blood. [42,43]

In the second Sherman study (2012), in which HIV RTs were performed on whole blood, the sensitivity of the Determine RT for the detection of HIV exposure in children <18 months of age and <3 months of age was 95.5% (95% CI 93.5-96.9) and 99.3% (95% CI 98.0-99.8) respectively which was similar to the findings in the present study of 94.7% (95% CI 92.3-96.4) and 98.7 (95% CI 96.2-99.6) respectively. [42] However, the sensitivity of the Insti RT was considerably lower in the present study (72.8% (95% CI 68.6-76.5) and 94.9% (95% CI 91.3-97.0) in children <18 months and <3 months of age respectively) compared to the Sherman study (95.7% (95% CI 92.4-97.6) and 98.7% (95% CI 96.2-99.6). [42] This is likely to be a reflection of the larger sample size in the present study.

Regarding specificity for exclusion of HIV exposure in children <18 months of age, the present study found Determine to have a lower specificity (93.1% (95% CI 90.1-95.2) than was found in the Sherman 2012 study (99.2% (95% CI 95.6-99.9) reflecting a higher rate of false-positive Determine RT results in the present study. For the Insti RT, a slightly higher specificity was found in the present study (99.3% (95% CI 97.9-99.8) compared to the Sherman study (96.2% (95% CI 87.0-98.9)). [42]

The seroreversion rate for the Determine RT at 8-10 months of age was 41.2% (7/17) in the present study versus 81.8% (9/11) in the Sherman 2012 study despite the

sensitivity of the Determine RT for detection of HIV exposure being similar in the 2 studies. Regarding the Insti RT, the seroreversion rate was 100% (17/17) at 8-10 months of age in the present study but there no data available in this age category in the Sherman 2012 study. However, in the 6-8 month age category, the seroreversion rate for the Insti RT was 77.6% (45/58) in the present study versus 100% (2/2) in the Sherman study. [42] Reasons for the different seroreversion rates between the 2 studies include the small sample sizes in these age categories, particularly in the Sherman study, and the possibility that in the present study (conducted between 2011-2014) there may have been greater infant exposure to maternal cART during pregnancy or breastfeeding than in the 2012 Sherman study. Maternal cART during pregnancy has been associated with delayed seroreversion among HIV-exposed but uninfected children. [15]

The ability of the Determine RT to detect HIV-exposed and infected (HIV ELISA+ HIV PCR+) children <18 months of age was 100% both in the present study (65/65) and in the De Baets study (116/116) but 93.1% (54/58, 4 HIV-infected children missed) in the study by Menzies et al. and 96.3% (105/109, 4 HIV-infected children missed) in the Sherman 2012 study [41,42,46] One of the differences between the present study and the De Baets studies on the one hand and the Menzies and Sherman 2012 studies on the other hand is that the HIV prevalence rate in the <18 month age category differs: 9.6% and 8.5% versus 25.6% and 18.8% respectively. This suggests that HIV-infected children <18 months of age are more likely to be missed using HIV antibody tests for screening in settings with higher HIV prevalence. Interestingly, of theses 4 studies, only the current study included children with acute illness attending or admitted in a paediatric hospital, a setting in which one may expect lower HIV antibody levels to be present in the child's circulation, but despite this no HIV-infected children were missed by the Determine RT. In the present study, the Insti RT missed 1 HIV-exposed and –infected child (1/68, 1.5%) and in the Sherman 2012 study, Insti detected 100% (32/32). [42]

Among children ≥18 months of age, the sensitivity and specificity results of the HIV RTs for the detection of HIV infection were comparable with other published studies with some variation with differing HIV prevalence rates in the study populations.

The sensitivity of the Determine RT was 100% in the present study (HIV prevalence rate 4.9%) and in the De Baets and Sherman (2012) studies (HIV prevalence rates of 3.9% and 64% respectively). The specificity of the Determine RT was 100% in the present study and in the De Baets study, and 97.5% (95% CI: 87.1-99.6) in the Sherman 2012 study (HIV prevalence 64%). [41,42] The sensitivity of the Insti RT was 92.3% (95% CI: 66.7-98.6) in the present study (HIV prevalence 4.9%), and 100% in the Sherman study (HIV prevalence 43%) while the specificity was 100% in the present study and 93.8% (95% CI: 71.7-98.9) in the Sherman study. [42]

Strengths and limitations

The present study has a number of strengths and limitations as indicated in the STARD checklist (Appendix 4). [63] The diagnostic accuracy of 6 HIV RTs used for HIV testing of children in South African health care facilities was rigorously evaluated in 2 accredited, quality-assured hospital laboratories thereby meeting the stated aims and objectives of the study. At each of the 2 study sites, a single experienced laboratory technologist performed all study HIV RTs according to the manufacturer's instructions. Whole blood rather than stored plasma or serum samples were used in the study so that the results obtained would be more generalizable to HIV testing contexts where centrifugation facilities are not available and whole blood samples are used for HIV rapid testing. Results of testing using the Determine RT on plasma were also available from the routine HIV testing laboratory for a subset of children at the CT study site and this allowed comparison of sensitivity and specificity performance between plasma and whole blood. The study design was prospective, based on the use of leftover blood samples from children with a wide range of ages that were in the process of undergoing HIV testing at the time, and as a result was efficient and cost saving. This avoided the need to recruit and enrol individual children onto the study, obtain informed consent for study participation from parents or legal guardians, or to subject children to venesection purely for study purposes. The reporting of this study followed the 2015 STARD methodology, a published international consensus approach to maximise the completeness and accuracy of reporting diagnostic accuracy studies. [63] The study was able to propose evidence-based recommendations on the future clinical role of

the index tests (HIV RTs) that were evaluated for HIV testing of infant and young children.

The limitations of this study have been identified by using the 2015 STARD checklist (Appendix) and relate mostly to the study design and setting. [63] The study design was based on the use of leftover blood from children undergoing routine HIV testing at 2 different study sites. Since the study did not enrol individual study subjects, recruitment of study samples depended on the number of children undergoing HIV testing at the 2 study sites, the submission of appropriate samples to the laboratory by clinicians, and availability of adequate volumes of leftover blood to use for performing the HIV ELISA test and HIV RTs. The study had no influence over which children underwent HIV testing and at what ages. The duration of the study was based on a convenience sample and funding availability.

The rate of recruitment of leftover blood samples was considerably slower than was anticipated. At the CT study site, this was as a result of very small blood volumes being submitted to the laboratory by clinicians at RCWMCH and the common practice of requesting the laboratory to perform HIV testing on an EDTA blood sample that was previously submitted for a full blood count. In response to the slow recruitment of adequate volume samples it was decided to include the use of plasma or serum leftover from biochemistry assays on the same child at the same time as the HIV test request in order to perform the study HIV ELISA test when required, and this improved the availability of adequate volumes of EDTA sample for the study HIV RTs to a certain extent. At the Jhb study site, inadequate blood sample volumes were responsible for many of the invalid HIV ELISA and HIV RTs.

Withdrawal of the SD Bioline RT by the SA NDOH during the course of the study and the replacement with the Abon RT resulted in a lower number of samples being evaluated with these 2 HIV RTs.

HIV PCR testing was not routinely performed on the children who tested negative with the routine Determine RT at the CT study site, as this is not included in the routine HIV testing algorithm and the CT study was not funded to allow HIV PCR

testing on all children. Lack of HIV PCR test results (21% of children <18 months of age) impacted on the calculation of HIV prevalence rate and seroreversion rate, and assessment of the ability of the HIV RTs to detect HIV-exposed and –infected children. In addition, the performance of the 6 HIV RTs for the detection of HIV-infected children was evaluated in comparison to a single positive HIV PCR result for children <18 months of age and a single positive laboratory HIV ELISA result for children ≥18 months of age. These were pragmatic definitions of HIV infection adopted for this study but do not concur with standard definitions of HIV infection that requires 2 positive HIV PCR tests in children <18 months of age and 2 positive HIV ELISA (or HIV RT) tests in children ≥18 months of age on 2 separate samples. [9,28-31]

Among children <18 months of age, there was a wide discrepancy in the sample size of younger children (<9 months of age) compared to older children (9-18 months of age) undergoing HIV testing. This partly reflects the widespread access to EID with HIV PCR testing in South Africa. South African HIV treatment guidelines published during the time this study was performed recommended initiation of cART for all HIV-infected children <5 years of age. As a result there are relatively few children between 9 and 18 months of age whose HIV status is unknown and who still require HIV testing. Successful implementation of the Prevention of Mother To Child Transmission (PMTCT) programme has resulted in significant reductions in the number of HIV-infected infants and young children. [6] The lower than anticipated numbers of HIV-exposed and HIV-infected children in the 9-18 month age category resulted in statistical uncertainty regarding the analysis of seroreversion rates and the ability of the HIV RTs to detect HIV-exposed and infected children in this age group.

HIV testing of infants and children takes place in a wide array of clinical settings including primary care clinics and hospitals with different laboratory infrastructure and is performed in infants and children with different prevalence rates of HIV exposure and HIV infection who may be clinically well or who have severe disease. The setting of this study was hospital-based and therefore not likely to be

representative of all other HIV testing settings. This limits the generalizability of the findings of this study to similar settings.

There were some differences between the 2 study sites. At the CT study site, leftover blood samples were obtained from children undergoing screening for HIV exposure and HIV infection according to a clinical algorithm applied to a hospital environment whereas at the Jhb study site leftover blood samples were obtained from children undergoing HIV PCR or HIV VL testing. Samples from children ≥18 months of age were obtained almost exclusively from the CT study site. The prevalence of HIV exposure and HIV infection may have differed between the 2 study sites but since the data from the 2 study sites was combined into a single dataset for the statistical analysis, the prevalence rate of HIV exposure and HIV infection was not calculated separately for the 2 sites. Small sample sizes, particularly in the 9-18 month age group precluded meaningful separate statistical analysis of the data from each study site.

The study design did not make provision for the collection and/or verification of the following demographic data relating to each of the children whose blood samples were included in the study: clinical condition of the child, maternal HIV status, maternal cART, infant feeding modality (breastfeeding and/or infant formula feeding), and exposure of the infant or child to ARV prophylaxis or treatment. Based on the submission of a blood sample for routine HIV testing to the laboratory at RCWMCH or for HIV PCR testing at the Jhb study site it was assumed that the child was not currently receiving cART but some children, particularly infants <6 weeks of age and breastfeeding HIV-exposed children of all ages, may have been receiving nevirapine as prophylaxis against acquiring HIV infection. Exposure to ARVs in the form of infant nevirapine or via maternal ARVs excreted in breastmilk may have impacted on the detection of HIV antibodies by HIV ELISA and/or HIV RT as well as the HIV PCR test result in the infant. Reduced sensitivity of HIV PCR testing in infants receiving ARV prophylaxis or cART has been previously reported. [16-18,20,23,64]

CONCLUSION

Despite the importance of HIV virological tests in confirming HIV infection in children <18 months of age, HIV RTs have an important role in screening children <18 months of age for HIV exposure in order to detect which children require further virological testing and in which children HIV infection can be excluded without further testing. Among children ≥18 months of age, HIV RTs generally perform well in the diagnosis of HIV infection, as in adults.

This study has shown that the diagnostic accuracy of HIV RTs for the detection and exclusion of HIV exposure among children <18 months of age, the age at which seroreversion amongst HIV-exposed uninfected infants and young children occurs, and the ability to detect HIV-infected infants and young children <18 months of age differs between different HIV RTs.

The findings of this study support the current HIV testing algorithm adopted by the Western Cape Province Department of Health and RCWMCH which recommends the use of the Determine RT for the initial screening of a child with unknown HIV status and in whom the current maternal HIV status is unknown.

Ongoing research and evaluation of the performance of different HIV RTs among infants and young children is needed. This research should include breastfed HIV-exposed infants and include infants and young children with exposure to maternal cART during pregnancy and breastfeeding. HIV RT performance should also be further evaluated in health care settings where personnel other than trained laboratory technologists working in controlled laboratory conditions perform HIV RTs.

RECOMMENDATIONS

- Among the 6 HIV RTs evaluated in this study, the Determine RT is recommended as the preferred HIV RT for screening children <18 months of age as it showed the highest sensitivity in comparison to laboratory HIV ELISA and despite failing to detect 25 HIV-exposed children, including 3 HIV-exposed children <4 months of age, no HIV-infected children were missed (all 25 HIV-exposed children were HIV PCR negative). The sensitivity of the Determine RT was even higher on plasma compared to whole blood samples and where laboratory facilities are available plasma should be the preferred sample on which to perform HIV RTs for screening infants and young children.

- In addition to the Determine RT, the SD Bioline, Abon and Advanced Quality RTs can be recommended for screening children <3 months of age as all 3 showed sensitivity and specificity for detection or exclusion of HIV-exposed infants in this age group of >97% and >99% respectively and no HIV-infected infants were missed. It is important to bear in mind that not all the children in this study had HIV PCR testing performed and so it is possible that some HIV-infected infants and young children were not detected by some or all of the HIVRTs.

- Besides infants <3-4 months of age, routine screening of HIV-exposed infants is recommended at around 9 months of age. Although in this study all 6 HIV RTs detected all HIV-infected children aged 8-10 months, very small sample sizes result in significant statistical uncertainty about this result.

- Similarly, small sample sizes prevent a strong and generalizable recommendation to be made on the preferred HIV RT for early and accurate detection of seroreversion among HIV-exposed uninfected infants and young children. Seroreversion was detected earlier with the INSTI and Advanced Quality RTs (Insti missed only 1 HIV-infected child and the child was 3-4

months of age) and later with the Determine RT than with any of the other HIV RTs evaluated.

- Among children ≥18 months of age, the SD Bioline, Abon or Determine RT, may be recommended as initial screening tests for the diagnosis of HIV infection as all 3 were shown to be 100% sensitive in comparison to laboratory HIV ELISA. The Insti and Advanced Quality RTs did not achieve adequate sensitivity to allow them to be recommended as initial screening tests but these results do not concur with their performance in other evaluations and may reflect the small sample size in this study. Other than the Reveal RT that was found to have very low sensitivity, any of the other 5 HIV RTs can be recommended as confirmatory tests for HIV diagnosis in children ≥18 months of age.

- Current South African NDOH HIV guidelines do not specify which HIV RTs should be used for HIV screening in children <18 months of age. Based on this and other studies, the evaluation of the diagnostic accuracy of specific HIV RTs in infant and paediatric populations should be a pre-requisite for consideration of inclusion in NDOH HIV RT tenders and use in HIV testing programmes involving infants and young children.

APPENDIX 1

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6626 • Facsimile [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

04 May 2011

Sent via Internal mail & Email

HREC REF: 218/2011

DR J NUTTALL,
Paediatrics & Child Health
Paediatric Infectious Diseases Unit
Red Cross Children's Hospital

Dear DR NUTTALL,

PROJECT TITLE: THE PERFORMANCE OF HIV RAPID TESTS IN CHILDREN.

Thank you for submitting your new study to the Faculty of Health Sciences Human Research Ethics Committee

It is a pleasure to inform you that the Ethics Committee has formally approved the above-mentioned study.

Approval is granted until 15 May 2012

Please submit an annual progress report (FHS018) if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

A/PROF MARC BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.

The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

APPENDIX 2

Western Cape Government

RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL
REFERENCE: Application for Research
Thomas.Blake@pgwc.gov.za

DR J NUTTALL

Dear James,

This letter serves to inform you that the research you applied to conduct [HIV Rapid Test] Study has been approved.

Regards,

DR T. BLAKE
MANAGER: MEDICAL SERVICES
RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL
DATE: 29/3/12

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Private Bag X5, Rondebosch, 7700
www.capegateway.gov.za/health/your_gov/5881

APPENDIX 3

UNIVERSITY OF THE WITWATERSRAND - JOHANNESBURG

School of Pathology
Department of Molecular Medicine & Haematology
7 York Road, Parktown 2193 Telephone 011 7172561/2000

NATIONAL HEALTH LABORATORY SERVICES

PO Box 1038, Johannesburg 2000 Telephone 011 489 8505 /9000

Prof Cleaton-Jones
Human Research Ethics (Medical)
Committee of the University of the
Witwatersrand, Medical School
15th June 2012

Dear Prof Cleaton-Jones

Re: An additional project under the Research and Development (R&D) Programme in the Department of Molecular Medicine and Haematology, University of the Witwatersrand

The department of Molecular Medicine and Haematology currently holds blanket ethics approval (M09-06-88: co-ordinated by Prof Lesley Scott), for validation studies and the development of new diagnostic assays. This blanket approval covers projects that will make use of residual routine specimens received by the NHLS for routine diagnostic testing. Once the routine testing is complete, R&D has access to these specimens. No additional patient specimens are received for the R&D testing. To date this approval has been granted for projects such as CD4 validation, HIV viral load validation, serology validations, coagulation assay development etc.

We would like to add another R&D project to this list:

Project title: ***The evaluation of seven commercially available Rapid HIV test performance on whole blood in HIV-exposed infants and children to establish suitability for an HIV diagnostic algorithm in infants and children.***
In 2011, the South African Department of Health adopted a policy of exclusive breastfeeding in HIV-exposed infants. An evidence-based diagnostic algorithm to

detect postnatal transmission of HIV is yet to be defined. This study aims to provide evidence to support such an algorithm.

The project has two objectives:

1. To determine the seroreversion (i.e. loss of maternal HIV antibodies) rate for each Rapid HIV test in children to exclude HIV infection.
2. To determine the frequency of loss of HIV antibodies (resulting in false negative rapid tests) in HIV-exposed and infected infants due to early initiation of antiretroviral therapy. If these children were inadvertently tested and false negative rapid test results obtained, there life-saving antiretroviral therapy may be erroneously discontinued.

Residual specimen from samples sent to the department's laboratories for HIV PCR, Viral load and occasionally CD4 testing will be submitted for an HIV ELISA test (to act as a reference standard for comparison to the rapid test results) and seven rapid HIV tests. No additional samples from the patient will be required and any additional costs incurred will be for the study's account. No patient identifying details will be captured however, for the 2nd objective the date of antiretroviral therapy initiation (if captured) on the laboratory information system may be required to be recorded for the data analysis of test performance.

Investigators: The PI for this study is Prof Gayle Sherman from the department of Molecular Medicine and Haematology University of Witwatersrand Medical School. The rapid HIV tests will be performed by Ms Kapila Bhowan (registered technologist).

I am happy that this project complies with the departmental R&D global program and we request this project to be included in the R&D programme under ethics number (M09-06-88).

We look forward to your response.

Regards,

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Prof Cleaton-Jones
Human Research Ethics (Medical)
Committee of the University of the
Witwatersrand, Medical School
15th June 2012

Dear Prof Cleaton-Jones

Re: An additional project under the Research and Development (R&D) Programme in the Department of Molecular Medicine and Haematology, University of the Witwatersrand

The department of Molecular Medicine and Haematology currenty holds blanket ethics approval (M09-06-88: co-ordinated by Prof Lesley Scott), for validation studies and the development of new diagnostic assays. This blanket approval covers projects that will make use of residual routine specimens received by the NHLS for routine diagnostic testing. Once the routine testing is complete, R&D has access to these specimens. No additional patient specimens are received for the R&D testing. To date this approval has been granted for projects such as CD4 validation, HIV viral load validation, serology validations, coagulation assay development etc.

We would like to add another R&D project to this list:

Project title: ***The evaluation of seven commercially available Rapid HIV test performance on whole blood in HIV-exposed infants and children to establish suitability for an HIV diagnostic algorithm in infants and children.***
In 2011, the South African Department of Health adopted a policy of exclusive breastfeeding in HIV-exposed infants. An evidence-based diagnostic algorithm to detect postnatal transmission of HIV is yet to be defined. This study aims to provide evidence to support such an algorithm.

Theproject has two objectives:
1. To determine the seroreversion (i.e. loss of maternal HIV antibodies) rate for each Rapid HIV test in children to exclude HIV infection.
2. To determine the frequency of loss of HIV antibodies (resulting in false negative rapid tests) in HIV-exposed and infected infants due to early initiation of antiretroviral therapy. If these children were inadvertently tested and false negative rapid test results obtained, there life-saving antiretroviral therapy may be erroneously discontinued.

APPENDIX 4

The Standards for Reporting Diagnostic Accuracy (STARD) Checklist

Section and topic	Item	Item reported in this study (page number)
Title or abstract	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values or AUC)	Yes (1)
Abstract	Structured summary of study design, methods, results, and conclusions	Yes (8)
Introduction	Scientific and clinical background, including the intended use and clinical role of the index test	Yes (12)
	Study objectives and hypotheses	Yes (43)
Methods		
Study design	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	Yes (41)
Participants	Eligibility criteria	Yes (44)
	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	Yes (44)
	Where and when potentially eligible participants were identified (setting, location, and dates)	Yes (44)
	Whether participants formed a consecutive, random or convenience series	Yes (46)
Test methods	Index test, in sufficient detail to allow replication	Yes (44)
	Reference standard, in sufficient detail to allow replication	Yes (43)
	Rationale for choosing the reference standard (if alternatives exist)	Yes (74)*
	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	Yes (47)
	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Yes (47)
	Whether clinical information and reference standard results were available to the performers/readers of the index test	Yes (45)
	Whether clinical information and index test results were available to the assessors of the reference standard	Yes (45)
Analysis	Methods for estimating or comparing measures of diagnostic accuracy	Yes (48)
	How indeterminate index test or reference standard results were handled	Yes (48)
	How missing data on the index test and reference standard were handled	Yes (48)
	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	Yes (48)
	Intended sample size and how it was determined	Yes (46)
Results		
Participants	Flow of participants, using a diagram	Yes (57)
	Baseline demographic and clinical characteristics of participants	No

	Distribution of severity of disease in those with the target condition	No
	Distribution of alternative diagnoses in those without the target condition	No
	Time interval and any clinical interventions between index test and reference standard	No
Test results	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Yes (60)
	Estimates of diagnostic accuracy and their precision (such as 95% confidence interval)	Yes (60)
	Any adverse events from performing the index test or the reference standard	No
Discussion		
	Study limitations, including sources of potential bias, statistical uncertainty, and generalizability	Yes (73)
	Implications for practice, including the intended use and clinical role of the index test	Yes (77/78)
Other information		
	Registration number and name of registry	No
	Where the full study protocol can be accessed	No
	Sources of funding and other support; role of funders	Yes (46)

* This is discussed as a limitation of the study in the Discussion section not the Methods section

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